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The Inhibition of a Diphosphopyridine Nucleotide System by Split Products of Dimethylaminoazobenzene*

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A study has been reported previously from this laboratory (5) of the diphosphopyridine nucleotide (coenzyme I) content of rat livers during the production of hepatic cancer by the oral administration of dimethylaminoazobenzene (butter yellow). The results showed that the diphosphopyridine nucleotide (DPN) concentration was decreased in the damaged livers and was exceedingly low in the neoplastic tissue. The decrease of the concentration of diphosphopyridine nucleotide in the tissue affected by dimethylaminoazobenzene made it desirable to establish whether that compound or its metabolites affect, *in vitro,* a system whose activity depends on diphosphopyridine nucleotide, and in which the latter is the limiting factor.

Kinosita (6) suggested that animals fed dimethylaminoazobenzene may excrete aniline and dimethyl-*p*-phenylenediamine in the urine. Stevenson, Dobriner, and Rhoads (17) (Fig. 1) have provided evidence that the butter yellow molecule is split by the rat *in vivo* at the azo linkage and they isolated *p*-phenylenediamine (both free and acetylated) and aminophenol (both free and conjugated) from the urine of rats fed dimethylaminoazobenzene. No dimethyl-*p*-phenylenediamine was found in the urines to which no sodium hydrosulfite had been added. Hence, in the animal, complete demethylation may occur with the excretion of *p*-phenylenediamine only.

MATERIALS AND METHODS

The diphosphopyridine nucleotide system employed was essentially that described by Myrback (13) and later by Axelrod and Elvehjem (1). The optimum pH, salt, glucose, and hexosediphosphate concentrations for fermentation were determined for the yeast apozymase used (No. 2040 Standard Brands, Inc.). The following system was employed in standard Barcroft-Warburg manometers.

After thermal equilibrium had been attained, the contents of the side arm were tipped into the main vessel and fermentation was measured for 1 hour. The amount of carbon dioxide produced in the first hour is a linear function of the amount of diphosphopyridine nucleotide added between 0 and 40 μgm. (Fig. 3). In the following experiments, 15 μgm.[1] of diphosphopyridine nucleotide were added to this system unless otherwise indicated.

As the methods originally available for the preparation of the apozymase yielded irregular and unstable products, a better one was sought. The following procedure yields an apozymase which, when kept in the icebox, is stable for at least 4 months:

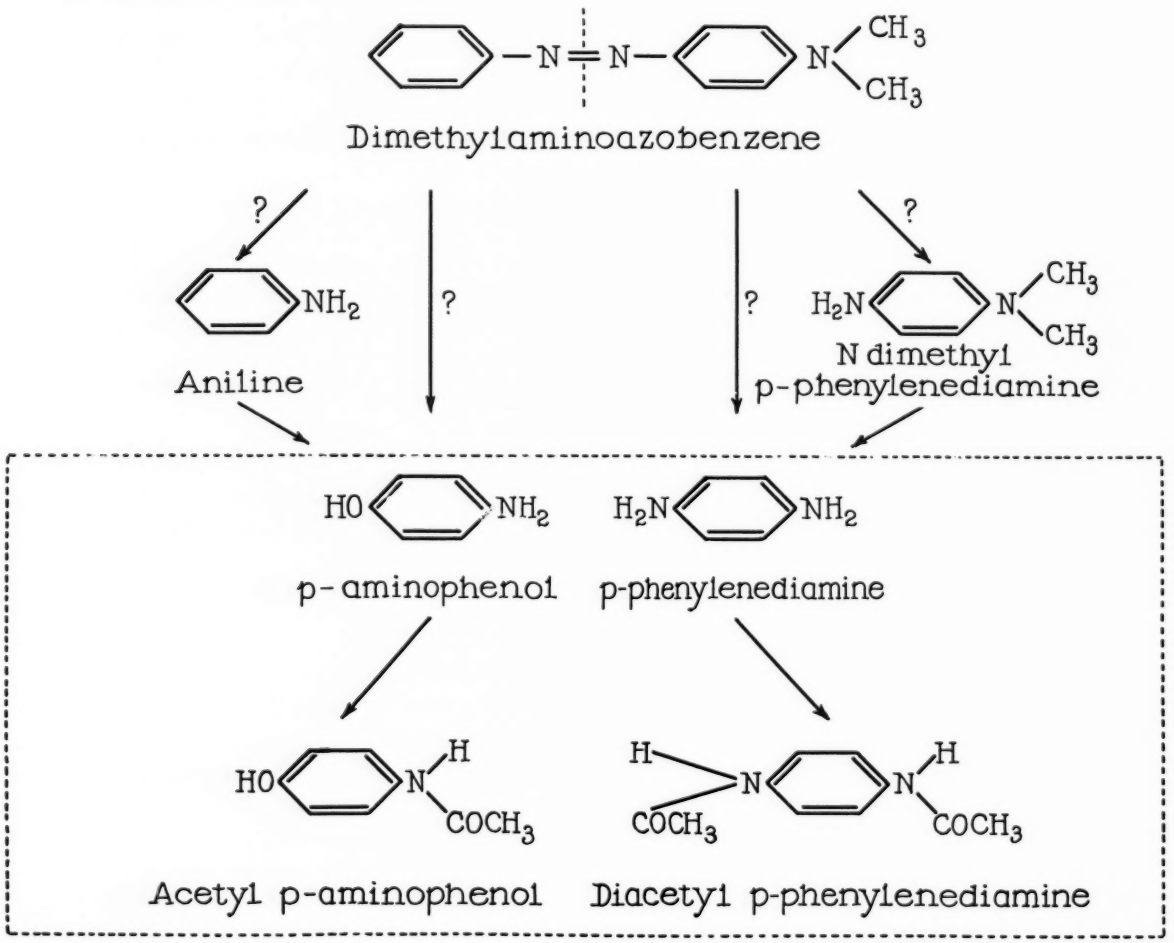
Five hundred gm. of No. 2040 yeast (Standard Brands), ground to pass through a 100 mesh screen, are washed with running tap water for 3 hours in a 12 liter short-necked, round-bottomed flask. The tap water is run through a piece of glass tubing to the bottom of the flask and the rate of flow adjusted to that velocity which will keep the particles in constant agitation with minimal loss into the overflow cup. The washed yeast is then collected and the excess water removed by squeezing in a gauze sack. The soggy mass is then placed in a vacuum freezing desiccator. Through the courtesy of Dr. James B. Murphy, we were allowed to use a desiccator in which the temperature was −23.3° C., the vacuum 2 to 3 mm. of mercury, and the desiccant calcium chloride. In this apparatus, drying was complete in 7 to 10 days. When dry, the material is ground to pass through a No. 40 screen and stored in the icebox. As it was impossible to remove all of the diphosphopyridine nucleotide from this yeast without inactivation of the whole fermenting complex, the figure for the residual activity, as read in the blank, was subtracted from the figure for the total carbon dioxide produced.

* This investigation was aided by grants from Standard Brands, Inc., and from The Jane Coffin Childs Memorial Fund for Medical Research.

† Deceased November 25, 1939.

[1] This value is based on the determination of the maximum absolute purity of the sample. This assay was made in collaboration with Dr. P. Handler and Dr. W. J. Dann of Duke University Medical School. They will report this experiment in a publication which will appear in the *Journal of Biological Chemistry.*

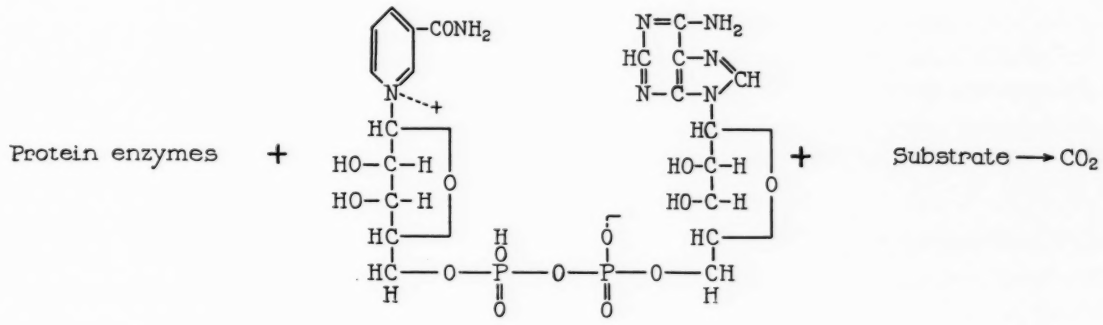
Metabolism of dimethylaminoazobenzene by the rat



Compounds within dotted lines have been isolated from the urine by Stevenson, Dobriner and Rhoads (17)

FIG. 1

Fermentation system



In main vessel		Tipped in from side arm
300 mg. apozymase	+3.0 cc. DPN solution in	+0.25 cc. 16% solution of glucose
+0.5 cc. 2% phosphate buffer pH 6.4	1% phosphate buffer pH 6.5	+0.15 cc. sodium hexosediphosphate solution (15 mg.)
+0.1 cc. 16% solution $MgCl_2 \cdot 6H_2O$		

V_f = 4.0 cc., Gas phase = air, T = 30°C.

FIG. 2

RESULTS

Effect on fermentation in the yeast system of dimethylaminoazobenzene, its metabolites, and related compounds.—Fermentation in the system described was not inhibited when the solution was saturated with dimethylaminoazobenzene. However, N,N-dimethyl-*p*-phenylenediamine (a potential intermediary metabolite) and *p*-phenylenediamine, isolated from the urine of rats fed dimethylaminoazobenzene (17), were found to cause a pronounced inhibition at concentrations of less than 5 x 10^{-4} molar. These two aromatic diamines are much more toxic than are quinone, aniline, *p*-aminophenol, or hydroquinone. The effects of the related compounds tested on the fermenting system are summarized in Table I, and it is clear from the results that the aromatic paradiamines were the most toxic. Since the unsymmetrical N-dimethyl derivative caused a greater inhibition than did the unsubstituted *p*-phenylenediamine, several other of its alkyl derivatives were tested.

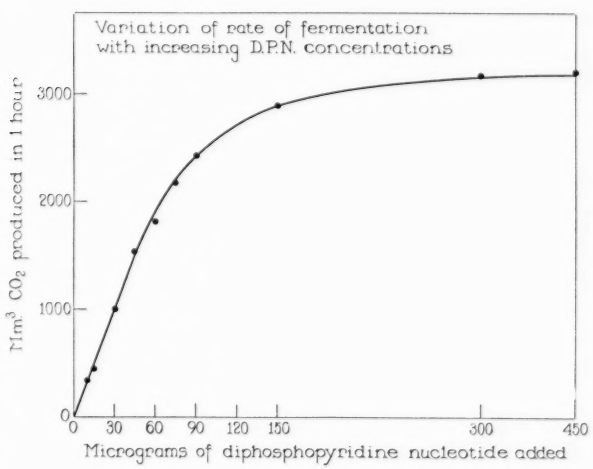


FIG. 3

TABLE I: EFFECT OF VARIOUS COMPOUNDS ON FERMENTATION OF DIPHOSPHOPYRIDINE NUCLEOTIDE SYSTEM

Compound	Molar concentration	Per cent inhibition
N,N-Dimethylaminoazobenzene	Saturated solution	10
Aniline	8.0×10^{-4}	15
p-Aminophenol	2.3×10^{-4}	6
N,N-Dimethyl-*p*-aminophenol	1.6×10^{-4}	5
N,N-Dimethyl-*p*-phenylenediamine	1.8×10^{-4}	100
p-Phenylenediamine	2.3×10^{-4}	75
Phenol	8.0×10^{-4}	25
p-Cresol	7.0×10^{-4}	0
Hydroquinone	2.2×10^{-4}	0
Catechol	2.2×10^{-4}	0
Naphthoresorcinol	2.5×10^{-4}	0
β-Naphthylamine	2.1×10^{-4}	20
1-Amino-2-naphthol	2.8×10^{-4}	28
2-Amino-1-naphthol	2.8×10^{-4}	0
Methylene blue	5.0×10^{-4}	0

Dr. Leonor Michaelis had previously prepared a large number of alkyl derivatives of *p*-phenylenediamine as a part of a general study of two-step oxidations,

and he very kindly provided samples of these compounds to test in the fermenting system. Many of these were also toxic, several more so than the unsymmetrical N,N-dimethyl-*p*-phenylenediamine possibly formed by the animal from the carcinogen dimethylaminoazobenzene. The results of these experiments are summarized in Fig. 4 and show a direct correlation between the toxicity of the compounds and the degree of stability of the free radicals (semiquinones) formed from them by partial oxidation. Free radicals of the type of Wurster's salts, and the factors which influence their stability, are discussed in detail in the publication by Michaelis, Shubert, and Granick (12).

Significance of oxidation in relation to the toxicity of the Wurster type compounds.—As is shown in Fig. 4, there is a definite correlation between the inhibition and the stability of the free radical of the inhibiting compound. The unsymmetrical N,N-dimethyl-*p*-phenylenediamine, which is capable of forming a very stable free radical, completely inhibits fermentation in the system employed. If a methyl group is inserted in the 2- position on the ring of this compound (ortho to the N-dimethyl group), the free radical formed is very unstable. At a concentration equivalent to that of the N-dimethyl compound, the 2-methyl compound was not toxic to the fermenting system. If the methyl group is substituted in the 3- or meta position on the ring, the free radical is somewhat less stable than that formed from N,N-dimethyl-*p*-phenylenediamine. The toxicity of the meta-methyl compound is also somewhat less than that of the unsubstituted N,N-dimethyl-*p*-phenylenediamine. As is indicated in Fig. 4, only those compounds which are capable of forming free radicals with a stability of more than 4 hours are markedly toxic to the system studied.

In addition to the correlation between toxicity and free radical stability, there is also, as shown in Fig. 4, a general correlation between the toxicity, and the rate of oxidation in the presence of the apozymase, of the compounds tested. Those compounds which form relatively stable free radicals were oxidized rapidly, whereas those which form very unstable free radicals, or none at all, were oxidized very slowly, if at all. The rate of oxidation in the presence of the apozymase was determined by the measurement of oxygen consumption. These measurements agree with the speed of formation of the colored oxidation production of the compounds tested, as estimated visually. The correlations between toxicity and free radical stability, as well as between toxicity and the relative ease of oxidation, suggest that the inhibition of fermentation in the diphosphopyridine nucleotide system employed is due to the formation of the free radical or possibly to some further oxidation product of the compounds tested. These steps in oxidation are shown in Fig. 5.

It is known that the quaternary diimonium compounds derived by further oxidation from the methylated aromatic paradiamines of the type tested are extremely unstable in aqueous solution (12). According to Willstätter, the diimonium compound derived from N,N,N′,N′-tetramethyl-*p*-phenylenediamine readily decomposes in the solid state with the formation of formaldehyde. As the decomposition of these diimonium compounds might produce quinone, monomethylamine, dimethylamine, and formaldehyde in the test system employed, these compounds were also tested.

Quinone was the only one which showed significant toxicity (Table II) and, furthermore, the addition of monomethylamine and formaldehyde did not increase the inhibition of the system to which quinone had been added. The inhibition caused by quinone, in a concentration equivalent to that which might be formed by the complete oxidation of N,N-dimethyl-*p*-phenylenediamine in the test system employed, is only 50 per cent as large as that caused by the N-dimethyl compound. Hence, all the toxicity of the N-dimethyl compound cannot be due to the formation of quinone. The fact that the inhibition of the diphosphopyridine nucleotide system by quinone was prevented by cysteine, while that due to N,N-dimethyl-*p*-phenylenediamine was not, also tends to eliminate quinone as a major toxic factor. As the concentration of the diimonium compound will be small due to its own instability and to the stability of the free radical from which it is formed by further oxidation, the free radical or its dimerization products are probably the only oxidation products which attain a significant concentration.

Table II: Elimination of Final Oxidation Products as Toxic Agents

Compound	Molar concentration	Per cent inhibition
Quinone	5.0×10^{-4}	51
Quinone + methylamine + formaldehyde	5.0×10^{-4}	51
Methylamine	1.0×10^{-3}	9
Dimethylamine	5.0×10^{-4}	0
Formaldehyde	1.0×10^{-3}	9
N,N-Dimethyl-*p*-phenylenediamine	1.8×10^{-4}	100

It is known that hydrogen peroxide is produced as a result of the auto-oxidation of many compounds. In order to determine whether or not the inhibition in the experiments here reported could be due to the formation of hydrogen peroxide by the auto-oxidation of the Wurster type salts, it was added to the system. There was no inhibition of fermentation (Table III) by an amount of hydrogen peroxide equivalent to that which might be formed from the oxidation of the reduced diamines (Table II). Furthermore, the addition of a catalase preparation (active in the catalysis of

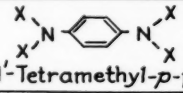
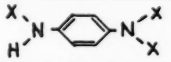
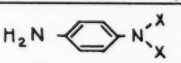
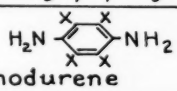
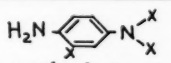
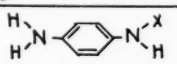
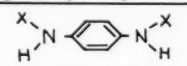
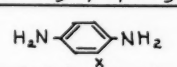
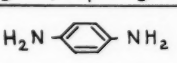
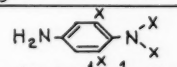
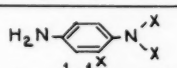
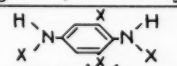
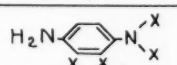
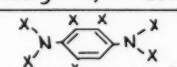
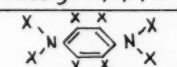
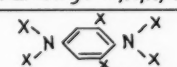
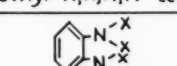
Compound	Molar concentration	Percent inhibition	Free radical stability(12)*	Oxidation by 200 mgm. apozymase in 15 min. ** cu. mm. O_2
 N,N,N′,N′-Tetramethyl-*p*-phenylenediamine	1.5×10^{-4}	100	weeks	35
 N,N,N′-Trimethyl-1,4-phenylenediamine	1.7×10^{-4}	70	7 days	38
 N,N-Dimethyl-*p*-phenylenediamine	1.8×10^{-4}	57	7 days	23
 Diaminodurene	1.5×10^{-4}	62	2–3 days	40
 2-Methyl-N^4,N^4-dimethyl-1,4-phenylenediamine	1.7×10^{-4}	50	2 days	18
 N-Methyl-*p*-phenylenediamine	2.1×10^{-4}	42	1 day	23
 N,N′-Dimethyl-*p*-phenylenediamine	1.8×10^{-4}	37	1 day	22
 2-Methyl-1,4-phenylenediamine	2.1×10^{-4}	35	4–8 hours	24
 *p*-Phenylenediamine	2.3×10^{-4}	23	4–8 hours	23
 2,6-Dimethyl-N^1,N^1-dimethyl-1,4-phenylenediamine	1.2×10^{-4}	0	2 hours	10
 2-Methyl-N^1,N^1-dimethyl-1,4-phenylenediamine	2.3×10^{-4}	0	5 min.	0
 2,6-Dimethyl-N^1,N^4-dimethyl-1,4 phenylenediamine	1.2×10^{-4}	0	1 min.	0
 2,3-Dimethyl-N^1,N^1-dimethyl-1,4-phenylenediamine	1.3×10^{-4}	0	0	0
 2,3,5,-Trimethyl-N,N,N,N^1-tetramethyl-1,4-phenylenediamine	1.2×10^{-4}	0	0	0
 2,3,5,6-Tetramethyl-N,N,N′,N′-tetramethyl-1,4-phenylenediamine	1.1×10^{-4}	0	0	0
 2,6-Dimethyl-N,N,N′,N′-tetramethyl-1,4-phenylenediamine	1.3×10^{-4}	0	0	0
 N,N,N′,N′-Tetramethyl-1,2-phenylenediamine	1.5×10^{-4}	0	0	0

FIG. 4.—Per cent inhibition of fermenting system by compounds of the type of Wurster's salts. Compounds were added as HCl salts.

* Michaelis' free radical stability determinations were made between pH 3.6 and pH 6.0. All of these free radicals are less stable above pH 6.0 (12). This experiment was run at pH 6.4.

** Concentration of compounds is M/200.

X = CH_3.

the decomposition of hydrogen peroxide) to the system did not prevent the inhibition by the Wurster type compounds. The catalase preparation itself had no effect on the system.

TABLE III: ELIMINATION OF H_2O_2 AS TOXIC AGENT

System	Mm. CO_2 in 1st hour
Control	715
Control and *p*-phenylenediamine (2.3×10^{-4} molar) and catalase	85
Control and catalase	725
Control and hydrogen peroxide (5×10^{-4} molar)	710
Control and *p*-phenylenediamine (2.3×10^{-4} molar)	75

The evidence suggests, by the elimination of other possibilities, that the toxicity of the Wurster type compounds tested is due to the formation, by oxidation, of free radicals in the fermenting system. To test further the suggestion that an oxidation product of these compounds is the toxic agent, the effect of several

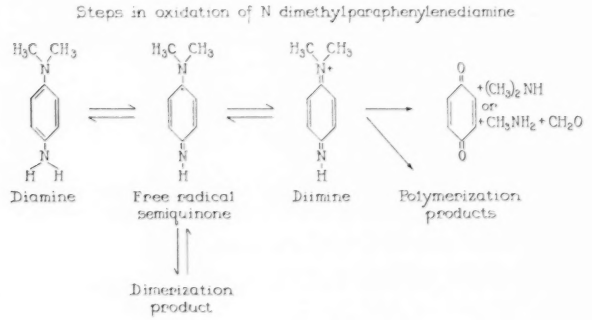


FIG. 5

reducing agents such as cysteine, glutathione, and ascorbic acid was tested by their addition to the system before the addition of the toxic substance (Table IV). The reducing compounds employed prevented the inhibition of the diphosphopyridine nucleotide system by *p*-phenylenediamine (Fig. 6) and quinone; furthermore, the inhibition appeared to be reversible. Even if the apozymase was incubated for 15 minutes with *p*-phenylenediamine before the addition of the cysteine, the reducing substance still prevented the inhibition. No such effect of cysteine was noted when N,N-dimethyl- and N,N,N′,N′-tetramethyl-dimethyl-*p*-phenylenediamine were employed. However, cysteine and glutathione in phosphate buffer pH 6.4 will keep both the N,N-dimethyl- and the N,N,N′,N′-tetramethyl compounds reduced, but when these compounds were added to the fermenting system, their oxidation was not prevented by cysteine or glutathione. The color of the free radical could be seen even though as much as 3 mgm. of cysteine had been added.

TABLE IV: EFFECT OF THE ADDITION OF REDUCING AGENTS ON THE INHIBITION OF DIPHOSPHOPYRIDINE NUCLEOTIDE SYSTEM BY THE WURSTER'S SALTS

System	Mm. CO_2 in 1st hour
Control	715
Control and *p*-phenylenediamine (2.3×10^{-4} molar)	75
Control and *p*-phenylenediamine and cysteine (3 mgm.)	645
Control and *p*-phenylenediamine and glutathione (3 mgm.)	690
Control and *p*-phenylenediamine and ascorbic acid (3 mgm.)	580
Control and quinone (5×10^{-4} molar)	345
Control and quinone and cysteine (3 mgm.)	605
Control and cysteine (3 mgm.)	700
Control and ascorbic acid (3 mgm.)	695
Control and cystine (3 mgm.)	520
Control and N,N-dimethyl-*p*-phenylenediamine (1.8×10^{-4} molar)	0
Control and N,N-dimethyl-*p*-phenylenediamine and cysteine (3 mgm.)	40
Control and N,N-dimethyl-*p*-phenylenediamine and glutathione (3 mgm.)	70

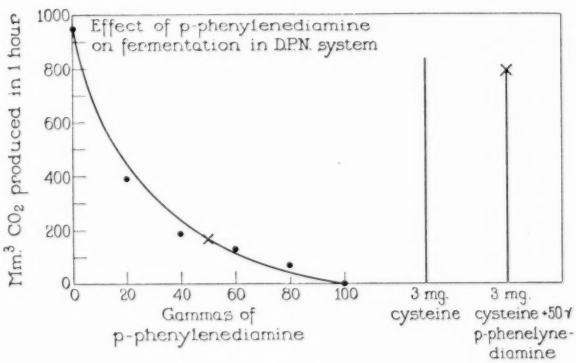


FIG. 6

Mechanism of the inhibition of fermentation in this system by the Wurster type compounds.—Dixon and Zerfas (2) have recently reported that alloxan is capable of acting as a hydrogen acceptor for certain dehydrogenases which had been supposed specifically to require diphosphopyridine nucleotide. This observation suggested the possibility that in the fermentation system employed in this study, in which the nucleotide is the limiting factor, alloxan might successfully compete with the coenzyme for the dehydrogenase and thus inhibit fermentation. As is shown in Table V, alloxan was very toxic to fermentation when low concentrations of diphosphopyridine nucleotide were employed. If the inhibition is due to alloxan competing successfully for the dehydrogenase, the percentage of inhibition should be much lower at higher coenzyme concentrations, and, as is indicated in Table V, this is actually the case (92 per cent to 7 per cent). These results indicate that diphosphopyridine nucleotide and alloxan are competing for an enzyme essential to fermentation, or in other words, that the high concentra-

tion of the nucleotide protects the enzyme and prevents its inactivation by, or the formation of an inactive complex with, alloxan.

The inhibition of fermentation by alloxan was similar to that by N,N-dimethyl-*p*-phenylenediamine in that neither was prevented by the addition of cysteine or glutathione to the system. As the experiments with the compounds of the Wurster series indicate that they owe their toxicity to the formation of a toxic intermediary oxidation product, these oxidation products may be competing successfully, as does alloxan, with diphosphopyridine nucleotide for the active dehydrogenase centers. As is shown in Table V, the inhibition by N,N-dimethyl-*p*-phenylenediamine behaves in a manner analogous to that by alloxan. The percentage inhibition is large at low concentrations and small at high concentrations of diphosphopyridine nucleotide.

TABLE V: EFFECT OF INCREASING AMOUNT OF DIPHOSPHOPYRIDINE NUCLEOTIDE ON PER CENT INHIBITION OF FERMENTATION

	Fermentation	Per cent inhibition
System + 15 μgm. DPN *	790	
System + 300 μgm. DPN	2,750	
System + 525 μgm. DPN	3,130	
System + 15 μgm. DPN + N,N-dimethyl-*p*-phenylenediamine (2.4×10^{-4} molar)	20	97
System + 300 μgm. DPN + N,N-dimethyl-*p*-phenylenediamine (2.4×10^{-4} molar)	2,500	9
System + 15 μgm. DPN + alloxan (2.2×10^{-4} molar)	65	92
System + 525 μgm. DPN + alloxan (2.2×10^{-4} molar)	2,895	7
System + 15 μgm. DPN + iodoacetate (5×10^{-4} molar)	0	100
System + 300 μgm. DPN + iodoacetate (5×10^{-4} molar)	0	100

DPN = Diphosphopyridine nucleotide.

However, in the presence of the high concentration of diphosphopyridine nucleotide, it was noted that color formed by the oxidation of the reduced diamines rapidly disappears when the contents of the side arm are tipped into the main vessel. As has been previously mentioned, this reduction does not occur when only 15 μgm. of the nucleotide are present. In general, actively fermenting systems possess strong reducing properties, and the system employed in these experiments is able to reduce the oxidized forms of the Wurster compounds. As is indicated in Table V, a portion of the inhibition by N,N-dimethyl-*p*-phenylenediamine is not overcome even when three times more diphosphopyridine nucleotide is present than is necessary to attain the maximum fermentation rate. The simplest interpretation of this phenomenon is that the inhibition is a competitive one and that the reduction of the oxidized Wurster type salts is a secondary process.

Insofar as is known, the role of diphosphopyridine nucleotide (DPN) in fermentation may be summarized by the following reactions:

1. Diphosphoglyceraldehyde + DPN → phosphoglyceric acid + reduced DPN (catalyzed by triosephosphate dehydrogenase)
2. Acetaldehyde + reduced DPN → ethyl alcohol + DPN

In addition, Meyerhof and co-workers have shown that this reaction is coupled with a phosphorylation step, as is indicated by the following equation:

1a. Phosphoglyceraldehyde + DPN + adenosinediphosphate → phosphoglyceric acid + reduced DPN + adenosinetriphosphate

The triosephosphate dehydrogenase which catalyzes reaction 1 has been shown to be sensitive to oxidizing agents, oxidized glutathione, iodine, and monoiodoacetate. For example, when the enzyme (prepared from rabbit muscle) was freed of diphosphopyridine nucleotide by charcoal adsorption, Rapkine (15) showed that the dehydrogenase could be inactivated by oxidized glutathione or iodine. If the preparation was not freed of the nucleotide, inactivation was difficult. The inactivation was prevented by the addition of reducing agents such as hydrogen sulfide and cysteine. These observations on the sensitivity of the triosephosphate dehydrogenase are similar to those reported in this communication on the enzyme active in the fermentation system. F. Lipmann (10) has shown that iodine and quinone will inhibit glycolysis in muscle extracts. Gemmill and Hellerman (3) confirmed the observation of Lipmann that iodine was an inhibitor and showed that this inhibition was removed by the addition of reducing agents as cysteine, glutathione, and ascorbic acid. Lipmann has also found (11) that quinone will inhibit fermentation in a maceration extract of yeast. Lipmann's conclusion was that the oxidized form of an enzyme essential to glycolysis was inactive, while reduction allowed its glycolytic fermentation to proceed. The results of these experiments are generally explained on the basis of an oxidative inactivation of SH groups, although this point is not as yet completely established.

Iodoacetate, which is capable of oxidizing SH groups, has been found in the experiment here reported to be a very strong inhibitor in the fermenting system employed (Table V). Vassel (18) has found that N,N-dimethyl-*p*-phenylenediamine will condense with cysteine in strongly acid solutions in the presence of Fe^{+++} and $ZnCl_2$. As alloxan has been shown to combine with sulfhydryl groups (9), the inhibition of activity of the fermenting system employed by alloxan and by the Wurster type compounds may be due, though this is still unproved, to their combination

with or oxidation of the SH groups of the triosephosphate dehydrogenase.

Detoxification of split products of dimethylaminoazobenzene by acetylation.—As many aromatic amino compounds are excreted in the urine as acetyl derivatives, and as N-acetyl-*p*-phenylenediamine was isolated from the urine of rats fed dimethylaminoazobenzene (17), the inhibitory effect of the acetyl derivatives of *p*-phenylenediamine and dimethyl-*p*-phenylenediamine was studied. Neither of these derivatives is oxidized by the apozymase preparation or shows free radical formation. As would be expected from the evidence, and as is shown in Table VI, neither of these compounds was toxic to the diphosphopyridine nucleotide system. Hence, the acetylation clearly represents a demonstrable mechanism of detoxification utilized by the rat *in vivo*.

TABLE VI: EFFECT OF ACETYLATION ON TOXICITY

System	Mm. CO_2 in 1st hour
Control	488
Control and N,N-dimethyl-*p*-phenylenediamine	78
Control and N-acetyl-N′,N′-dimethyl-*p*-phenylenediamine	520
Control and *p*-phenylenediamine	95
Control and N-acetyl-*p*-phenylenediamine	488
Control and N,N-diacetyl-*p*-phenylenediamine	498

Concentration of compounds = 1×10^{-3} molar.

DISCUSSION

The two azo dyestuffs, orthoaminoazotoluene and dimethylaminoazobenzene, the carcinogenic properties of which are well established, are both split at the azo link in the course of their metabolism in the rat. Hashimoto (4), in 1935, reported this fact for orthoaminoazotoluene. His conclusion was based on the isolation of 2-methyldiacetyl-*p*-phenylenediamine from the urine of rats fed orthoaminoazotoluene. As mentioned previously, Stevenson, Dobriner, and Rhoads (17) have reported that dimethylaminoazobenzene is split at the azo link, but they were unable to find any free or acetylated N,N-dimethyl-*p*-phenylenediamine in the urine, although they did isolate free and acetylated *p*-phenylenediamine. It is assumed that N,N-dimethyl-*p*-phenylenediamine is formed in the body and then, by demethylation, is converted into the less toxic *p*-phenylenediamine.

If the production in the liver cells of toxic water-soluble aromatic paradiamines from the fat-soluble azo dyestuffs plays any role in carcinogenesis, it is reasonable to assume that there should be some correlation between carcinogenic potency and the toxicity of the aromatic paradiamines formed as split products of the carcinogen. As is shown in Fig. 7, the available information on the carcinogenic potency of various methyl derivatives of aminoazobenzene correlates rather well with the toxicity of the diamino split products. The recent experiment of Nagao and coworkers (14), in which the following methyl derivatives of dimethylaminoazobenzene were tested for carcinogenic potency, illustrates this point. The insertion of a methyl group (compound I in Fig. 7) in the free para position of dimethylaminoazobenzene (II) does not significantly decrease the carcinogenic potency of butter yellow. If a methyl group (III) is substituted in the position meta to the dimethylamino group of this molecule (I), the carcinogenic potency is reduced. The aromatic paradiamine split product from this molecule (III), 3-methyl-N,N-dimethyl-*p*-phenylenediamine, is less toxic in the diphosphopyridine nucleotide system than the unsubstituted N,N-dimethyl-*p*-phenylenediamine obtained from (I), and the free radical stability is significantly reduced. If a methyl group (IV) is inserted ortho to the dimethylamino group, the carcinogenic property of the molecule is completely lost. The 2-methyl-N,N-dimethyl-*p*-phenylenediamine split product of this molecule (IV) is not toxic in this diphosphopyridine nucleotide system, and the free radical form of this compound is very unstable. The reduced toxicity and low free radical stability of the split product from orthoaminoazotoluene (V) agree well with the comparatively low carcinogenic potency of this molecule. Aminoazobenzene (VI), which yields *p*-phenylenediamine as the split product, does not produce liver cancer in the rat.

If this correlation is more than a coincidence, and if other factors such as cell permeability, the rate of splitting of the azo link, and the rate of acetylation of the split products remain relatively constant, it should be possible to predict the carcinogenicity of other methyl derivatives of aminoazobenzene. If the aromatic paradiamine split product has a toxicity of greater than 65 per cent in the diphosphopyridine nucleotide system employed, and the free radical stability is more than 4 hours, the original compound would be expected to produce liver cancer in the rat. Conversely, those methyl derivatives of aminoazobenzene whose split products do not satisfy these conditions would not be expected to be carcinogenic.

As is shown in Fig. 7, the insertion of a water-soluble functional group on the butter yellow molecule renders the molecule noncarcinogenic (VII, VIII). This loss of carcinogenic property is also true of the hydrocarbon carcinogens when a water-soluble group is introduced into the molecule. The substitution of alpha or beta naphthylamine for the aniline portion of the dimethylaminoazobenzene molecule (IX) also decreases the carcinogenic potency.

The correlation between carcinogenic action on rat liver cells and the toxicity of the potential diamino split

products seems to apply only to methyl derivatives of aminoazobenzene.

Summary and Conclusions

1. A method of preparing a stable apozymase is described.

2. One metabolite (*p*-phenylenediamine) and a probable intermediary metabolite (N,N-dimethyl-*p*-enediamine and *p*-phenylenediamine are not toxic to the system.

5. Alloxan has also been found to be highly toxic to the system.

6. These experiments indicate that the inhibition of this system both by the Wurster type compounds and alloxan is a competitive one in which the substance competes with diphosphopyridine nucleotide for an enzyme active in fermentation.

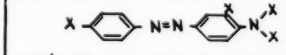
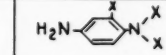
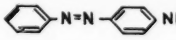
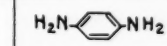
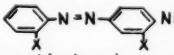
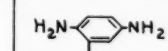
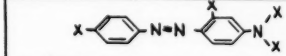
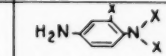
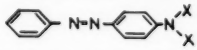
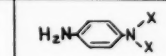
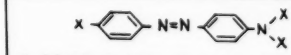
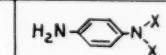
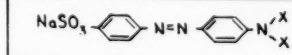
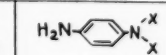
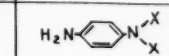
Compound	Diamine split product	Per cent inhibition of DPN system by diamine split product	Free radical stability of split product[12]	Carcinogenic potency (liver)	Reference
 4,5′-Dimethyl-N,N-dimethylaminoazobenzene IV		0	5 min.	0	Nagao (14)
 Aminoazobenzene VI		38	4-8 hours	0	Sasaki & Yosida (16)
 2,3′-Dimethylaminoazobenzene V		65	4-8 hours	+	Yosida (20)
 4,6′-Dimethyl-N,N-dimethylaminoazobenzene III.		83	2 days	+	Nagao (14)
 N,N-Dimethylaminoazobenzene I.		92	7 days	++	Kinosita (6)
 4-Methyl-N,N-dimethylaminoazobenzene II.		92	7 days	++	Nagao (14)
 VII. (Methyl orange) Sodium N,N-dimethylaminoazobenzene sulfonate		92	7 days	0	Kinosita (7)
 VIII 2-(N,N-Dimethyl-*p*-aminophenylazo)-naphthalene		92	7 days	±	Kinosita (7)
X = CH_3					

FIG. 7.—Compounds showing correlation between toxicity of diamine split products and carcinogenic potency (liver) of the parent molecule.

phenylenediamine) of N,N-dimethylaminoazobenzene, which produces hepatic cancer in rats and causes a decrease of the diphosphopyridine nucleotide content of the damaged livers, have been shown to be toxic in low concentrations to a fermenting system in which diphosphopyridine nucleotide is the limiting factor.

3. From a study of the metabolites and compounds related to them, it is concluded that the toxic effect is due to the formation of an intermediary oxidation product of the compound in the fermenting system.

4. The acetyl derivatives of N,N-dimethyl-*p*-phenyl-

7. The evidence suggests that the triosephosphate dehydrogenase is the enzyme which is inactivated by these compounds.

8. The apparent correlation between toxicity in this system of the diamine split products of methyl derivatives of aminoazobenzene and the carcinogenic potency for the rat liver of the parent molecule is discussed.

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Neurofibromas of Rat Ears Produced by Prolonged Feeding of Crude Ergot

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As a result of an experiment to determine the chronic toxicity of crude ergot when fed to our inbred strain of Osborne-Mendel albino rats for 2 years, 23 of 38 rats on a 5 per cent dosage developed multiple tumors up to 8 mm. in diameter on their ears. Tumors appeared also on the ears of 9 of 38 rats on a 2 per cent dosage, but not on any of 38 on a 1 per cent dosage.[1] Histologically these tumors are neurofibromas, some showing distinct palisading of nuclei. They have appeared only on the ears. Male and female rats are affected alike. Among several hundred of our rats allowed to live normally or subjected to other experimental procedures, and whose ages equal those of the rats fed ergot, only one fairly similar tumor has appeared; this tumor was also on an ear. The tumors induced by the feeding of crude ergot are not truly malignant, inasmuch as they will regress markedly within a few weeks if feeding of the drug is stopped. Should the feeding be resumed they will then regain their former size and histologic appearance in 2 or 3 months. If the feeding is not resumed, some of the tumors will begin a spontaneous new growth after about 6 months.

Materials, Methods, and Results

Source of ergot.—The ergot used by us was a mixture of a dozen or more lots of imported ergot pooled to give a total of about 40 pounds of crude ergot, which has lasted throughout the experimental work reported here. Crude ergot loses its activity very slowly, as measured either by the U. S. P. cock's comb method or by colorimetric assay for total alkaloids, if it is kept dry in a tightly covered container. Ground ergot deteriorates more rapidly (1), and therefore we ground small lots when needed.

Experimental procedure.—Groups of 20 rats each were started at 3 weeks of age on dosages of 5, 2, and 1 per cent of ground crude ergot in their diet; the rest of the diet consisted of ground dog-food pellets plus 1 per cent cod liver oil. Probably because it was not learned until later that rats would tolerate 5 per cent of ergot better if the dosage were increased gradually, 11 of this group of rats died within 1 year. Three other rats in this group died at 14, 18, and 22 months of age; then, in a rat dying at 23 months of age (in May, 1940; Pathology No. 308) it was noted that several soft nodules up to 4 mm. in diameter were present on the ears. On microscopic examination they were diagnosed as fibromas, most likely of perineural origin. The remaining 5 animals in the 5 per cent group, dying at greater ages or surviving the 2-year experimental period, all had similar tumors on the ears, with a similar microscopic appearance. It is quite possible that the 2 rats dying at 18 and 22 months of age had small ear tumors which had not been noticed. Of the groups of rats fed 2 per cent and 1 per cent of ergot, 16 of each 20 lived from 18 to 25 months; 3 rats on the 2 per cent dosage and none on 1 per cent had ear tumors.

While these experiments were in progress and before the ear tumors had appeared, a second series of experiments was begun, with the same levels of ergot in a low protein diet; there were 18 rats in each of these 3 groups. When the rats of the second series were 6 months of age, ear tumors were seen in the animals of the first series. When rats of the second series were 11 months of age, ear tumors began to appear in the group fed 5 per cent ergot; they continued to appear until at 17 months of age every one of the 17 living rats had ear tumors. Microscopic sections of these tumors showed the same neurofibromatous structure as in the first series. At the time of writing this report (October, 1941) tumors have also appeared on the ears of 6 rats in the 2 per cent group and on none in the group receiving 1 per cent ergot. These rats are now 23 months of age. The low protein diet of the second series of rats seems to have increased somewhat the size and number of the tumors and to have caused them to appear earlier than those in the first series.

* Read in part at the Forty-first Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 11, 1941.

[1] On November 19, 1941, when galley proof was returned, 2 rats on a 1 per cent dosage had developed small tumors on their ears, and one further tumor had appeared in the group on a 2 per cent dosage.

Gross appearance of tumors.—Grossly the tumors are rounded, circumscribed but not encapsulated, moderately firm subcutaneous masses on the inner, outer, or both surfaces of the ears. They are greatest in number at the periphery and least frequent toward the external meatus. The number on each ear varies from 1 to 8 (Fig. 1). On section the tumors are pinkish-white and rather pearly. The smaller tumors are uniform throughout; the larger ones often show ulceration and crusting of the surface (Fig. 2), and occasionally show internal areas of looser texture and slightly darker color than the rest of the tumor. The maximum size reached, after several months of growth, is 8 mm. or slightly greater in diameter. When an ear has several tumors of this size the tumors almost coalesce, and the aggregate size tends to remain fairly constant through ulceration and drying of portions of the tumors.

Routine microscopic sections of the major viscera of these rats, those without ear tumors as well as those with them, have shown that the tumors occur only on the ears, and that they do not metastasize.

Microscopic appearance of tumors.—The microscopic appearance of the tumors is quite uniform, and is characteristic of neurofibroma. The tumors are composed of oval to spindle-shaped cells of medium size, with leptochromatic nuclei and little neutrophilic cytoplasm, often arranged in interlacing whorls (Fig. 5) and fairly often in palisade formation (Fig. 3). Mitoses are few in number, varying from about 1 to 10 per square millimeter of tumor section. Small nerves containing both myelinated and unmyelinated fibers are frequent among masses of tumor cells (Fig. 4). The frequent presence of nerves within tumor tissue and of nuclear palisading leaves little doubt that these tumors are neurofibromas.

Further support of the microscopic diagnosis is given by various selective stains. The Masson and Van Gieson types of fiber stains show few collagenous fibers. With silver impregnation a dense feltwork of argentophilic fibers is seen. Frozen sections stained with Sudan IV show no fat in the tumor cells; a small amount is present in the macrophages in ulcerated areas. Sections stained with osmic acid show a similar picture. These macrophages also contain a small amount of brown pigment, some of which reacts for ferric iron (hemosiderin) with acid ferrocyanide. There is no pigment in the tumor cells.

The tumors will grow through the naturally occurring holes in the cartilage plate of the ear, and will also cause more or less pressure atrophy of the cartilage and of the adjacent striated muscle.

Regression and reappearance of tumors.—It occurred to us to try the effect of stopping the feeding of ergot to rats with well established tumors. Truly malignant induced internal tumors, such as the liver carcinomas resulting from the feeding of ortho-aminoazotoluene to rats, will keep on growing after the feeding of the carcinogen is stopped (5, 7). As no peripheral tumors other than the neurofibromas described in this report have as yet been produced by feeding a substance, we have no basis of comparison. The feeding of ergot was therefore stopped for 9 rats on the 5 per cent level, all of which had ear tumors. Three weeks later most of the tumors showed a marked reduction in size. After 3 more weeks there was a further slight reduction, so that the former typical tumors of about 8 mm. diameter were now about 4 mm. across and 1 mm. in height, while some of the smaller tumors had practically disappeared.

Six weeks after it had been discontinued, the feeding of 5 per cent ergot was resumed for 4 of the 9 rats. The tumors on the ears of these 4 rats remained about as described above for 2 months, but at the next examination a month later there had been marked growth, so that 3 months after the resumption of ergot feeding the tumors on 3 of the 4 were almost the size previously attained, while the fourth, which had small tumors to begin with, showed slight growth. Further growth took place later. Eventually some of the tumors were larger than before the animals had been taken off the 5 per cent ergot diet.

Meanwhile, the tumors on the 5 rats for which ergot feeding was not resumed showed a slow, progressive decline in size. At 5 months, partly because the larger remaining tumors had been removed for microscopic study, there remained only flat brown spots up to 3 mm. in diameter. These were best seen by transmitted light. However, at 6 months a new development was noted. Four of the 5 rats died about a week apart, and on an ear of one of these was a new 4 mm. tumor,

DESCRIPTION OF FIGURES 1 TO 5

FIG. 1.—Gross appearance of multiple and bilateral tumors on the ears of rats Nos. 849, 853, and 863.

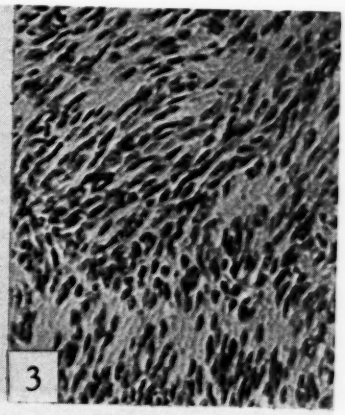
FIG. 2.—Photomicrograph of a longitudinal section through one of the larger tumors, from rat No. 1661, showing ulceration of the overlying epidermis, and a small extension of the tumor beneath the cartilage plate of the ear. Hematoxylin and eosin stain. Mag. × 18.

FIG. 3.—Photomicrograph of an area in a neurofibroma in rat No. 861, showing palisade arrangement of nuclei. Hematoxylin and eosin stain. Mag. × 225.

FIG. 4.—Photomicrograph of tumor in rat No. 830, showing a small nerve surrounded by tumor tissue. Masson type stain. Mag. × 575.

FIG. 5.—Photomicrograph of area in a tumor in rat No. 855, showing whorl formation and cellular details. Hematoxylin and eosin stain. Mag. × 750.

1



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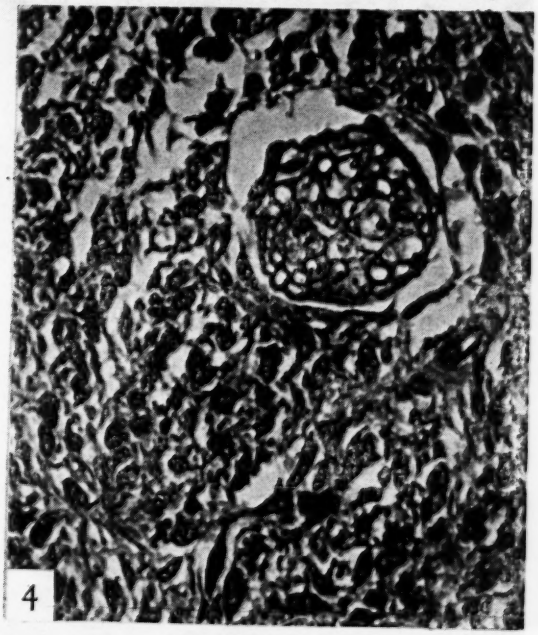

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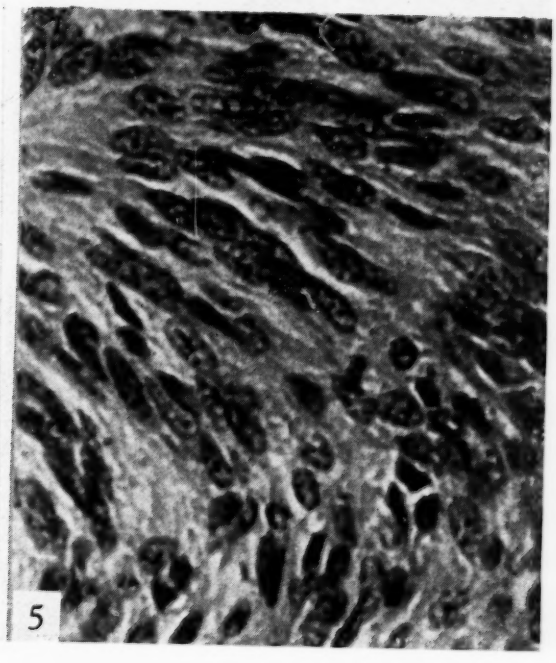

5

FIGS. 1-5

with the typical histologic appearance of these tumors. Also, the one rat still alive, after having had no ergot for 7 months, showed a new 2 or 3 mm. tumor. It would appear, then, that the residues of the tumors left after ergot feeding is stopped can grow spontaneously after a latent period of several months. The difficulty here is that the regrowth begins to take place near the end of the life span of the rat.

At intervals during the course of study of regression and reappearance of these tumors, several of them were removed for microscopic study. Three weeks after discontinuing ergot one tumor showed, in addition to the decrease in size, less active tumor tissue, in which were a moderate number of macrophages containing fat as shown with Sudan IV, and fewer containing brown pigment, chiefly hemosiderin as shown with acid ferrocyanide. At 4 months, in 5 tumors removed from 2 rats, various stages of regression were present. Two tumors were essentially as just described; another showed marked regression, appearing as a mass of loose, faintly mucoid fibrous tissue, with very little fat or pigment. The remaining two tumors, although small, had practically the same histological appearance as in the animals still on ergot. Collagen and reticulum stains in the regressing tumors showed these components to be present in about the same relative degree as before; the total amount was, of course, decreased. At 6 months, in the tumors showing no regrowth, there was only a little collagenous thickening, a little grayish-brown pigment in macrophages, and few or no tumor cells.

Rous and Kidd (8) have recently discussed the regression of tar tumors on the ears of rabbits when application of the tar is stopped.

Neurofibromas and Other Spontaneous Tumors in Rats Not Fed Ergot

In about 1,000 rats of all ages, not fed ergot, and studied histologically concurrently with those fed ergot, only one neurofibroma has occurred. This was on the ear of a rat 20 months of age and measured $7 \times 7 \times 3$ mm. Histologically it was slightly malignant and was classed as a grade 1 neurofibrosarcoma; it still retained the whorl formations of a neurofibroma. In the literature on rat tumors, both spontaneous and induced, we have been unable to find any diagnosed as neurofibroma or neurosarcoma. It is possible, however, that some of those diagnosed as fibroma or fibrosarcoma are in this class.

The feeding of ergot did not increase the incidence of the usual assortment of spontaneous tumors and leukemias to which the rat is subject, and which have been carefully studied for our colony. The rats fed ergot have had the usual number of these conditions, and no more, even among the group of animals bearing highly malignant fibrosarcomas which many believe have a neurogenic origin (2).

Pathological Changes Other than Tumor in Rats Fed Ergot

A report on the visceral lesions in these rats will be included later in a paper dealing with the pharmacological aspects of this study. To summarize briefly, however, it may be stated here that two other lesions specifically caused by the ergot were frequently observed. The first was necrosis and calcification of the lower end of the renal medulla, more marked in the groups on the low protein diet, although control animals on a low protein diet never had this lesion. We have never seen or read of this lesion in rats; it is found in some mice (3). The ovaries were frequently enlarged and mulberry-like, and composed chiefly of corpora lutea, similar to the appearance produced by chorionic gonadotropin; none of our other rats have shown this. Another, but nonspecific, lesion was a stunting of growth proportional to the dosage of ergot.

No vascular or cardiac lesions attributable to the ergot were found, and no gangrene occurred. Such experimental lesions as have been produced along these lines have usually been obtained by injection of ergotamine tartrate (6) in doses far greater than present in our crude ergot.

We have seen no report of the feeding of crude ergot for more than 3 months to animals other than roosters (4). A case of human cutaneous carcinoma in which the prolonged application of an ointment containing ergot played an uncertain part (9) has been described.

Further Investigation

Our findings raise a number of questions which our laboratory, not being primarily equipped for cancer research, cannot investigate in detail. The chief question concerns the exact fraction of ergot responsible for tumor production. Ergot is a highly complex substance. As space does not permit even a listing of its constituents, the reader is referred to Barger's (1) monograph. Tissue culture and transplantation have not been attempted, nor has ergot been fed to animals other than rats. We invite attempts by others to investigate these points. A rough fractionation study, with the feeding of defatted ergot, fatty residue, and ergotoxine to different groups of rats has been started by us.

Summary and Conclusions

1. Histologically typical neurofibromas have been produced on the ears, and on the ears only, of a high percentage of rats by prolonged feeding of 5 per cent of crude ergot in the diet.

2. The tumors have occurred less frequently on a level of 2 per cent of crude ergot and rarely on a level of 1 per cent. A low protein diet somewhat favors the production of tumors.

3. The neurofibromas have been made to regress markedly by withholding ergot, and then to reappear by refeeding.

4. After about 6 months without feeding of ergot, some tumors which have markedly regressed will spontaneously grow again; however, this is practically at the end of the life span of the animals.

5. Two other lesions, a renal medullary necrosis and calcification, and enlargement of the ovaries, are frequently caused by feeding of ergot. No cutaneous gangrene and no vascular lesions attributable to ergot have been observed, probably because the dosage in terms of alkaloids has been much too low.

6. The exact constituent of the crude ergot responsible for the tumor production is not known.

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Increased Viscosity of Cells of Induced Tumors*

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(Received for publication August 18, 1941)

In an earlier study (1) the authors found that tumor cells (carcinoma, sarcoma, adenofibroma, adenocarcinoma) of rats remained unstratified or stratified but feebly when centrifuged at extremely high speeds. In that series of experiments the tumor tissue was first transplanted to such organs as adrenal gland, kidney, pancreas, liver, spleen, stomach, and intestine. After growth was well established, bits of the transplant together with pieces of the host tissue were rotated in a Beams air-driven ultracentrifuge at a speed which produced a displacement pull of about 400,000 times that of gravity. Invariably the cells of the normal tissues became markedly stratified; those of the tumor tissue remained largely unchanged. It was inferred therefore that the protoplasm (both cytoplasm and karyoplasm) of the latter had become so viscous that the cellular contents were unable to move freely and take positions according to their respective densities.

In the present study advantage has been taken of the well-known tendency of butter yellow, *p*-dimethylaminoazobenzene, to induce cancer of the liver after prolonged feeding. The liver tissues used were from rats which had lived on a ration containing butter yellow for from 1 to $6\frac{1}{2}$ months.

We are indebted to Van Rensselaer Potter of the McArdle Cancer Research Institute for liver tissue from the 24 rats used in this study. After treatment was begun the animals were killed, 3 at a time, at the end of 4, 6, 8, 10, 13, 18, 21, and 26 weeks respectively. The photomicrographs (Figs. 1 to 7), illustrating the cytological effects observed, were made from paraffin sections of tissue fixed in Bouin's fluid and stained with Harris' hematoxylin and eosin or acid fuchsin.

The purpose of our investigation was to find out if the cells of such induced tumors showed increased viscosity in comparison with adjoining normal cells centrifuged at the same time, and if so, what could be determined about the onset of the condition.

For purposes of general histological guidance and comparison we depended mainly on Orr's (2) recent careful study on the histology of the rat's liver during the course of carcinogenesis by butter yellow. Of 136 rats fed on a diet containing butter yellow and killed at intervals through a period of from 1 to 11 months, Orr found that 56 displayed tumors, of which 43 were definitely malignant. The tumors described by him were of three types: (a) bile duct carcinomas (cholangiomas); (b) bile duct cystadenomas; and (c) liver cell carcinomas (hepatomas). Any particular liver might contain all three kinds or any one or two.

Occurrence of excessive proliferation of ducts and the presence of cystic bile ducts were usually the first indication of a definitely affected liver. Almost invariably, in the vicinity of such proliferative centers, certain areas of the liver cells lose their characteristic corded arrangement and become more or less nodular in appearance, although nonneoplastic liver cells may remain scattered throughout the developing tumor. Within such disorganized masses of cells, tubules or cysts begin to appear—first with small lumina and thick walls of columnar cells. As the tubules become fully developed the cells of the walls become cuboidal and eventually flattened. Such cells in all stages of

* This investigation was aided by research grants from The Jonathan Bowman Memorial Fund, The Brittingham Trust Fund, and from Elizabeth Hopkins Johnson.

DESCRIPTION OF FIGURES 1 TO 7

FIG. 1.—Photomicrograph of section of normal liver of the rat showing stratification of the component cells after centrifuging for 1 hour. The vacuoles below the nuclei represent regions originally occupied by glycogen which has been dissolved out by treatment with water. Mag. ×430, approx.

FIG. 2.—Photomicrograph of section of centrifuged normal liver stained for glycogen only. The glycogen has been displaced to the centrifugal side of the cell. Mag. × 430, approx.

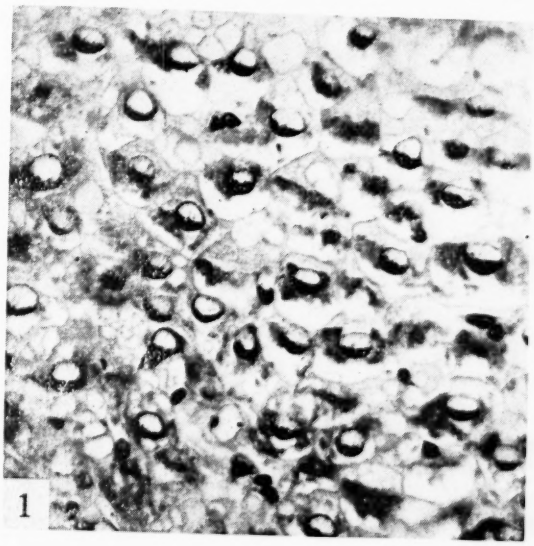
FIG. 3.—Photomicrograph of section of a nodule of the liver of the rat showing effects of administration of butter yellow. Mag. × 430, approx.

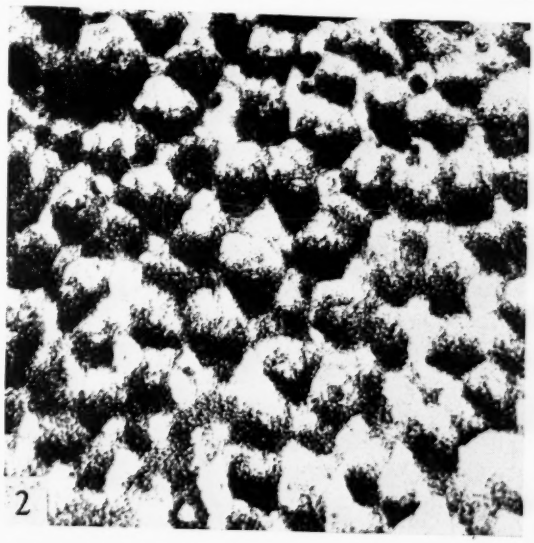
FIG. 4.—Photomicrograph of section of tissue from a nodule in the same liver as that from which the section shown in Fig. 3 was taken. After an hour of severe centrifuging the cells remain unstratified. Mag. × 430, approx.

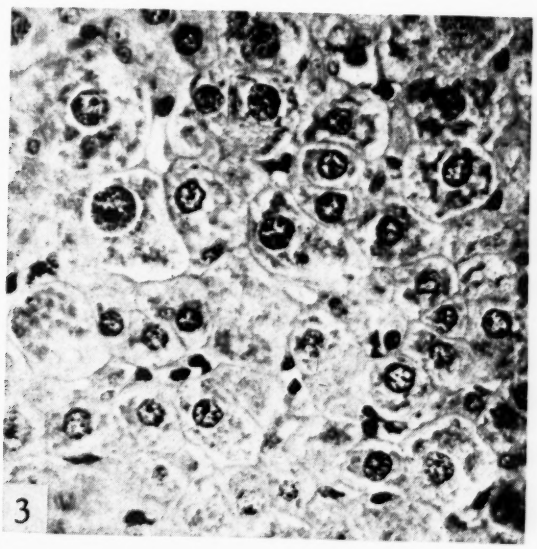
FIG. 5.—Photomicrograph of a representative area of centrifuged tissue from the liver of a rat after 6 months' treatment with butter yellow, showing proliferation of bile ducts, granulation, and pyknosis. Mag. × 100, approx.

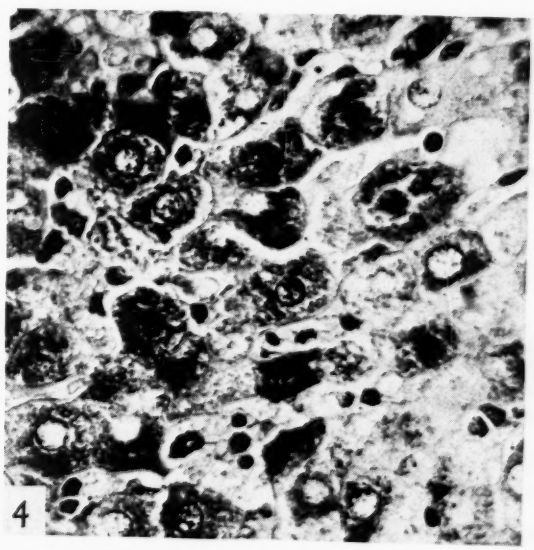
FIG. 6.—Photomicrograph of section of the same liver as that from which the tissue shown in Fig. 5 was obtained. After an hour of severe centrifuging the cells of the ducts and cysts remain unstratified. Mag. × 430, approx.

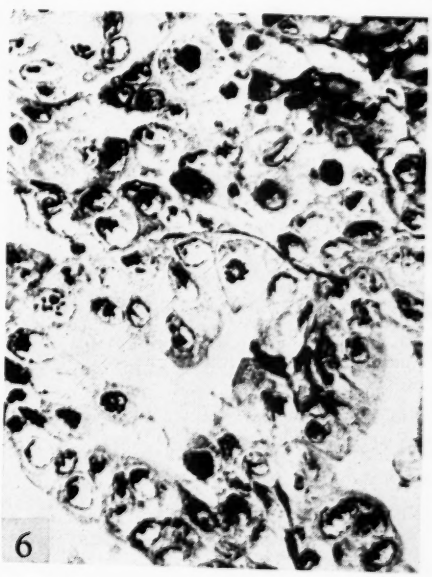
FIG. 7.—Photomicrograph of a section from a carcinomatous nodule in the liver of a rat after six months' treatment with butter yellow. Mag. × 430, approx.

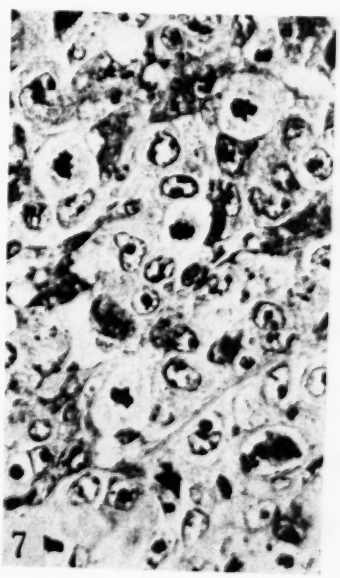












FIGS. 1 TO 7

their development remain unstratified after centrifuging at high speed for one hour (Figs. 4 and 6). In addition to the cystic systems just described, vacuolation of greater or less degree is often also present in the adjacent intercellular substance.

While they were obviously multifocal in origin, there was no way of determining whether the nodules just mentioned start as single cells or as groups of cells. The cells of the nodules are characterized by obvious variability in size with here and there much larger ones (Fig. 3). The nuclei of the latter are also enlarged and may contain several nucleoli instead of the usual single one. Moreover a greater number of cells than is usual in the liver cells of the rat display two nuclei. There is considerable pyknosis and the cells in general stain more deeply with the conventional hematoxylin and acid fuchsin dyes. In some preparations relatively few mitotic figures are observable; in others, they are plentiful (Fig. 7).

In liver cells of such foci (Fig. 3), centrifuging for an hour failed to produce appreciable stratification in either cytoplasm or nucleus (Fig. 4), although the non-neoplastic cells of the same liver tissue were markedly stratified (Fig. 1), with glycogen thrown to the extreme centrifugal side of the cell (Fig. 2) and nucleus surrounded by mitochondria next on top of it. The lighter cytoplasmic inclusions graded off toward the opposite side of the cell where a vacuole usually occurred. This vacuole evidently represented a region from which some lipoidal substance, probably the Golgi apparatus (1), had been dissolved by fat solvents in the later treatment of the sections. Within the nucleus of such normal cells the chromatin and the nucleolus were thrown into a crescentic mass against the nuclear wall with strands of achromatic substance stretching across to the opposite side.

In the livers of 3 rats fed on butter yellow for 2 months, where evidence of neoplasms was just becoming visible, (Fig. 3) the affected regions showed intensified staining indicating chemical change, and, when centrifuged, stratification did not occur (Fig. 4). Whether or not this indicates the onset of malignancy is impossible to decide surely. That it may be such is perhaps indicated by the occasional occurrence of areas containing scattered large cells. Longer feeding of butter yellow led to the occurrence of definite tumors the cells of which, like those of typical carcinomas, remain unstratified after being centrifuged. This was also true of the cells constituting the walls of the superabundant cysts and ducts (Figs. 5 and 6).

Normal liver cells centrifuged for 1 hour showed stratification (Fig. 1). Some of the centrifuged cells appear to show two vacuoles, one on the centrifugal (heavy) and one on the centripetal (light) side of the cell. This was puzzling for a time until similar cells were fixed in alcohol and stained specifically for glycogen. The centrifugal side of the cell was thus shown to be occupied by glycogen and the apparent vacuoles were obviously areas from which glycogen had been dissolved in the aqueous treatment of such cells. The vacuole on the lighter side of the cell was that left by dissolution of lipoidal substance, probably the Golgi apparatus, by fat solvents used in treatment of the sections. Glycogen is evidently the heaviest substance in the centrifuged normal liver cells stained for glycogen only (Fig. 2). According to Orr (2), glycogen does not occur in the cells of fully malignant liver cell carcinomas. The contents of cells comprising nodules in the liver of a rat which had been on a diet of butter yellow for 2 months (Fig. 3) were not stratified by centrifuging for one hour (Fig. 4). Similarly, the cells of proliferated bile ducts and cysts in the liver of a rat after 6 months of feeding with butter yellow (Fig. 5) were not stratified by severe centrifuging (Fig. 6).

Since it is now well established that the respiratory metabolism of cancer cells is so altered that the carbon dioxide output is largely replaced by formation of lactic acid, and with the well-known clotting effect of this acid on milk proteins in mind, one is prompted to inquire if the generation of excess lactic acid may not be the cause of the increased viscosity observable in cancer cells. At least the two phenomena are concurrent and it would seem worth while to determine, if possible, whether or not they are interrelated. Further investigations in this field are in progress.

Summary

High speed centrifuging of tumor cells induced in the livers of rats by the feeding of butter yellow (*p*-dimethylaminoazobenzene) leaves such cells unstratified. Normal liver cells stratify readily, with glycogen appearing heaviest, nucleus and mitochondria next, and lipoidal substances, mainly Golgi apparatus, lightest. Inside the normal nucleus of such cells the chromatin and nucleolus are forced to one side, with achromatic strands of material left stretching across to the opposite wall. The failure of either cytosome or nucleus of induced tumor cells to stratify when centrifuged along with adjacent normal liver cells is evidently due to the increased viscosity of their cellular contents. Such viscosity apparently comes on fairly early since it is evident in incipient tumor cells seen after some 2 months of feeding butter yellow. It is suggested that the increasing lactic acid output of the abnormal cell may be the cause of its enhanced viscosity.

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The Effect of Radioactive Phosphorus on the Viability of Mouse Sarcoma 180*

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Although artificial radioactivity was first induced but nine years ago by F. Joliot and I. Curie, at the present time about two hundred radioactive isotopes of the common elements are known. Some of these have been used satisfactorily for tracer-reaction studies and others have proved to be valuable materials for study of metabolism.

Some radioactive isotopes are selectively absorbed by certain organs and tissues. Thus radioactive iodine (1, 17-19, 22, 23) is selectively taken up by the thyroid gland; radioactive iron (12-14, 39) in the red blood cell; radioactive sodium (10, 11, 15, 16, 20) in the spinal fluid and the blood plasma; radioactive phosphorus (2-9, 21, 24-30, 32-36, 38, 40, 45-49) in the bones, liver, leukemic cells, and neoplastic tissues; and radioactive calcium and radioactive strontium (41, 42) in the skeleton. On the other hand, no detectable localization was noted following the administration of radioactive potassium and radioactive rubidium (16).

In view of the fact that the radioactive isotopes, in the course of disintegration, give off radiant energy (beta rays, gamma rays, positrons, etc.) similar to radium, the question at once arises as to what will be the effect of radioactive isotopes on tumor tissues; that is, whether the radiation of radioactive isotopes has effects on tumor tissue quantitatively different from those of x-rays or radium. This can be determined readily by exposing tumor fragments to the radioactive isotopes, and subsequently transplanting such fragments into host animals. The amount of reaction produced by a radioactive isotope is thus compared with that produced in tumors similarly treated with x-rays.

In the present study radioactive phosphorus was used because the radiations (beta rays) that are given off by it penetrate a considerable thickness of animal tissues, and P^{32} has a reasonably prolonged activity; *i.e.*, a half life time of 14.3 days.

MATERIALS, METHODS AND RESULTS

Mouse sarcoma 180 was selected on account of the regularity and high percentage of successful takes (essentially 100 per cent) obtained in transplantation experiments. The transplants showed only occasional spontaneous regression (about 4 per cent).

Subcutaneous inoculations of the tumor fragments in the lateral thoracic region of healthy young adult albino mice (Rockland albino mice) were carried out by the usual trocar method, the tumor materials being selected from rapidly growing tumors which had not ulcerated. These were from 7 to 10 days old. Aseptic precautions were taken throughout.

For the study of the effect of radioactive phosphorus upon mouse sarcoma 180 we used 8 preparations, containing respectively 286, 517, 850, 475, 265, 202, 260, and 192 microcuries (μc.) per cc. at the time they were used.

The radioactive phosphorus was prepared by bombarding red phosphorus with deuterons in the cyclotron at the Crocker Radiation Laboratory,[1] University of California, and thereafter was converted to a neutral solution of sodium phosphate.

Our previous studies with transplantable carcinoma and sarcoma (43, 44) indicated that these tumors showed marked differences in their reaction to different hydrogen-ion concentrations and salts in different concentrations. Because of possible changes in the reaction of the P^{32} solutions on shipping and standing we determined the hydrogen-ion concentration of these solutions electrometrically. It was found that the P^{32} solutions had pH values of 7.15 to 7.40. At these pH levels, the growth capacity of mouse sarcoma 180 was unaffected.

Since the P^{32} solution contained 15 mgm. of Na_2HPO_4 per cc., the extent of the deleterious action of disodium hydrogen phosphate upon the growth of mouse sarcoma 180 in mice was determined. It was found that immersion of fragments of mouse sarcoma 180 in 1.5 per cent Na_2HPO_4 solution (pH 7.4) for from 24 to 48 hours, at 4-5° C., was without effect, the tumors subsequently growing normally when implanted in animals. This study included three groups of experiments, involving a total of 40 implants.

* Acknowledgment is made to the Works Projects Administration of New York City for assistance rendered under Project 24.

[1] The author is indebted to Dr. John H. Lawrence of the University of California for the radioactive phosphorus used in this investigation.

Effect of P^{32} on the Growth of Mouse Sarcoma 180

Various concentrations of P^{32} were prepared by diluting the original phosphate solutions with 1.5 per cent ordinary Na_2HPO_4 solution (pH 7.4). The solutions had previously been sterilized in a steam autoclave at 15 pounds pressure for 15 minutes.

Eleven pieces of fresh tumor tissue (mouse sarcoma 180), each weighing about 6 mgm. and measuring 1.5 to 2.0 mm. in thickness, were placed in 2 cc. portions of P^{32} solution. The weighing bottles (approximately 14 mm. in diameter) containing the tumor fragments were kept in a refrigerator for 24 hours at 4-5° C. with gentle shaking four times in 12 hours.

Table I: Results of Transplanting Mouse Sarcoma 180 after Immersion in Radioactive Phosphorus Solution at 4-5° C.

Experiment number	Number of tumor transplants	P^{32} (μc./cc.)	Duration of immersion (hours)	Equivalent roentgens	Growth of transplants (per cent)
1	10	40.0	24	1,720	100
2	10	49.9	24	2,140	100
3	10	57.4	24	2,460	100
4	10	61.7	24	2,650	90
5	10	63.2	24	2,710	100
6	10	65.0	24	2,790	90
7	10	76.5	24	3,280	70
8	10	84.3	24	3,620	80
9	10	87.4	24	3,750	50
10	10	92.5	24	3,970	70
11	10	97.5	24	4,180	70
12	10	99.8	24	4,280	60
13	10	113.0	24	4,850	40
14	10	114.0	24	4,890	40
15	10	115.0	24	4,930	40
16	15	120.0	24	5,150	60
17	10	126.0	24	5,400	10
18	10	131.0	24	5,620	30
19	10	143.0	24	6,130	0
20	10	151.0	24	6,480	0
21	10	160.0	24	6,860	0
22	10	164.0	24	7,040	0
23	10	216.0	24	9,270	0

At the end of this period of time, the tumor fragments were removed from the P^{32} solution to a Petri dish containing a sheet of semi-moist filter paper and were immediately implanted into mice by the usual trocar method, each animal receiving a single graft.

The results obtained from these experiments are presented in Table I.

The data in Table I show clearly that the growth capacity of mouse sarcoma 180 was not altered when tumor fragments were immersed for 24 hours at 4-5° C. in a solution of P^{32} of about 50 μc. per cc., the takes and growths being the same as in untreated controls. The inhibiting action, however, sharply increased with the increase in concentration of P^{32}. Thus immersion in a solution containing 75 μc. of P^{32} per cc. resulted in about 25 per cent inhibition and slight delayed growth. Marked inhibition and retardation of growth were caused by immersion in P^{32} solutions of 100 and 125 μc./cc. (about 50 and 75 per cent inhibition respectively). The viability of the tumor was completely destroyed by immersing in P^{32} solution of 150 μc./cc.

It may be of interest to mention that under the stated conditions of irradiation, the beta rays emitted from the radioactive phosphorus have not induced any stimulating influence upon mouse sarcoma 180.

Histological examinations of a number of the tumor tissues were made after exposure to P^{32}. The general structure of nonirradiated tissue showed a very cellular, small spindle and polyhedral cell sarcoma, in areas degenerated and necrotic. The sections of irradiated tissues showed no definite changes, the tumor cells appearing to be viable. Yet the growth energy of the transplants which had been immersed in P^{32} solution of 143 μc./cc. or more for 24 hours at 4-5° C., was completely destroyed.

Comparison with Effects of X-Rays

The lethal effect produced by beta rays of P^{32} on a transplantable mammalian tumor was compared with that produced by 200 kv. roentgen rays. With the method used by Marinelli (37), it is possible to calculate the beta-ray tissue dose received by the tumor fragments in the radioactive phosphorus solution in terms of "equivalent roentgens."

The beta particles produced by the disintegration of P^{32} have an average energy of 700 kv. and can penetrate between 2 and 4 mm. of animal tissue. According to Marinelli's calculation, if the beta ray energy of 1 μc. of P^{32} is released in a gram of tissue during a 24 hour period, 42.9 equivalent roentgens will be delivered to that tissue. By using the conversion figure, the *maximum* equivalent roentgen values for given microcuries were obtained as shown in the fifth column of Table I. These are maximum values based on the energy absorbed by the solution itself. The tumor fragments received lesser doses for two reasons: (a) Each was not necessarily surrounded by enough solution of P^{32} to receive a full complement of the beta radiation. (b) The P^{32} concentration, within the tumor fragment, was zero at the beginning of the immersion period, and therefore initially the tumor cells in the fragment could receive only beta rays originating in the solution outside of the fragment. As P^{32} diffused into the tissue, the cells received additional beta radiation from points in the fragment itself. The contribution to the total radiation received by the tumor cells from this P^{32} increased gradually with time. It

reached a maximum when no further diffusion of P^{32} took place. This point, however, may not have been reached during the period of immersion (24 hours). We know, however, that after this period the concentration of P^{32} in the tissue is about 60 per cent of that in the surrounding solution.

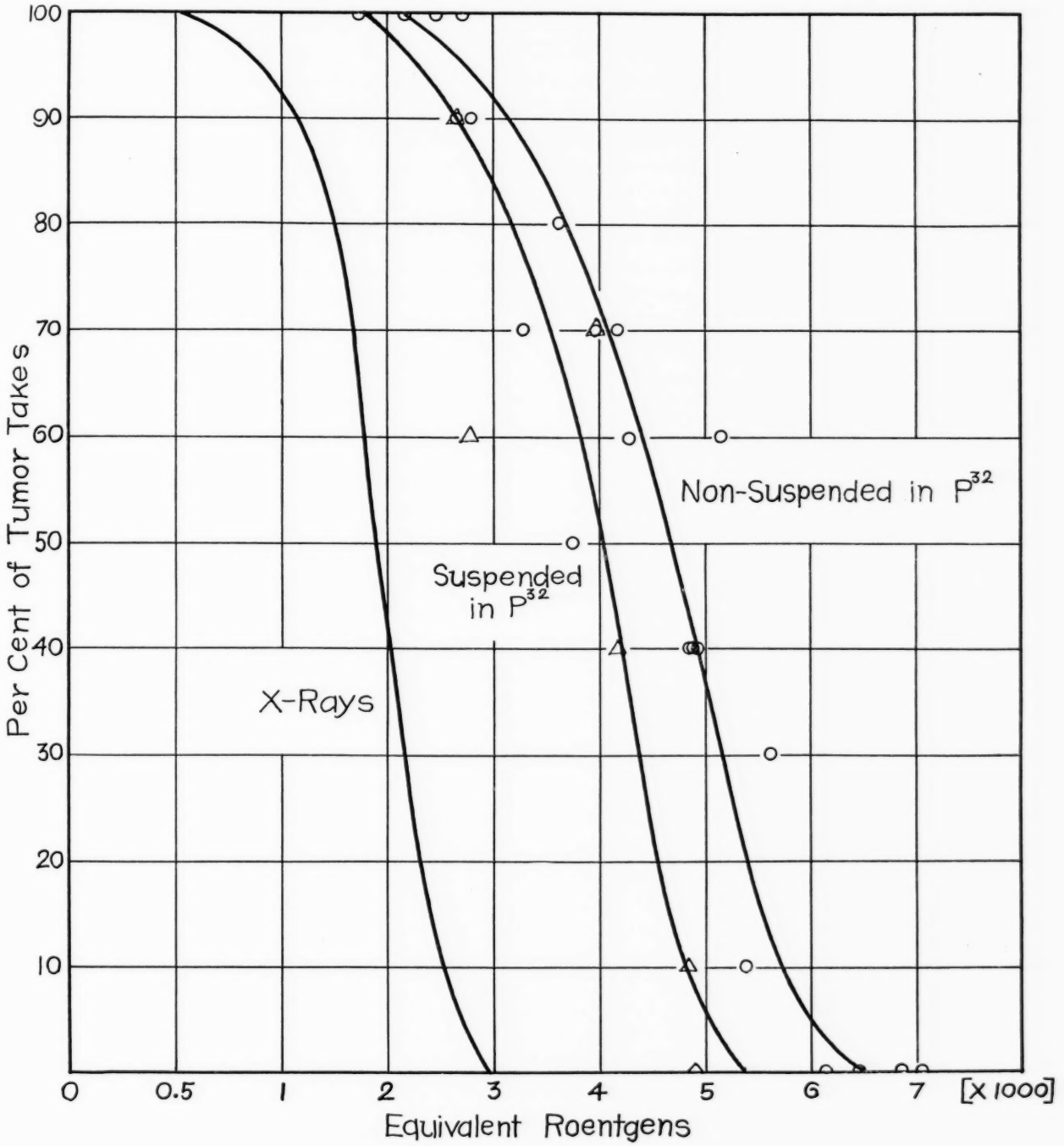

FIG. 1.—Survival curves of mouse sarcoma 180 exposed to beta rays from radioactive phosphorus and to x-rays. "Nonsuspended" fragments rested on bottom of bottle.

As may be seen from the data in Fig. 1, the transplantability of mouse sarcoma 180 was not altered appreciably by irradiation of tumor fragments with 2,500 equivalent roentgens (about 100 per cent takes). An exposure of 3,500 equivalent roentgens gave about 20 per cent inhibition. Marked inhibition was caused by an exposure of 4,500 and 5,500 equivalent roentgens (about 50 and 80 per cent inhibition, respectively). The viability of the tumor was completely destroyed by an exposure of 6,500 equivalent roentgens.

In the present experiment the tumor fragments were immersed in P^{32} solution at the bottom of the weigh-

ing bottle. Initially some tumor fragments fell side by side, thus possibly preventing full effect of beta rays emitted from P^{32}. Therefore, in order to give tumor tissues maximum radiation effect the tumor fragments were placed in a wide mesh gauze bag and suspended in the radioactive solution. Parallel experiments were run with tumor fragments immersed in P^{32} solution in the usual manner. After standing for 24 hours at 4-5° C. the suspended and nonsuspended tumor fragments (those lying on the bottom of the bottle) were implanted into mice. The results are presented in Table II.

The results of these comparative experiments show that tumor fragments suspended in P^{32} solution gave definitely fewer takes than those not suspended in P^{32} solution; *i.e.*, there was about 30 per cent increase in tumor destruction. Of course, in the case of the suspended fragments, irradiation is from all sides, whereas those on the bottom of the bottle received little radiation on their lower sides.

Table II: Results of Transplanting Tumor Fragments Suspended and Nonsuspended in P^{32} Solution for 24 Hours

Experiment number	Number of tumor transplants	P^{32} (μc./cc.)	Equivalent roentgens	Condition of tissue exposure	Growth of transplants (per cent)
1	10	61.7	2,650	Suspended	90
	10	61.7	2,650	Nonsuspended	90
2	10	65.0	2,790	Suspended	60
	10	65.0	2,790	Nonsuspended	90
3	10	92.5	3,970	Suspended	70
	10	92.5	3,970	Nonsuspended	70
4	10	97.5	4,180	Suspended	40
	10	97.5	4,180	Nonsuspended	70
5	10	113.0	4,850	Suspended	10
	10	113.0	4,850	Nonsuspended	40
6	10	114.0	4,890	Suspended	0
	10	114.0	4,890	Nonsuspended	40

Absorption of P^{32} by Tumor Tissue

In the course of the investigation a study was made on the extent of absorption of P^{32} by the tumor tissue. Twenty small pieces of tumor tissue, each weighing about 6 mgm., were placed in 2.0 cc. portions of P^{32} solution of 86 μc./cc. The weighing bottles containing the tumor fragments were kept in a refrigerator at 4-5° C. for 6, 24, and 48 hours. At the end of these time intervals, the tumor fragments were removed from the bottles, dipped into a Locke-Ringer solution for a second, blotted on filter paper, and then weighed and ashed in an electric furnace at 500° C. Afterwards the ashes were measured for beta-ray activity, using standard electroscopic methods. Tissue ash so measured was found after 6 hours soaking to yield 46 μc./gm., after 24 hours, 53 μc./gm., and after 48 hours, 60 μc./gm. It is to be noted that after the first 6 hours of soaking in phosphate solution, the tissue exhibits a P^{32} concentration which is about 53 per cent of the P^{32} concentration of the soaking solution; and that after 24 hours the P^{32} concentration within the tissue has risen to 62 per cent. It is evident that tumor fragments so treated and implanted into mice carry with them a considerable quantity of radioactive phosphorus, which continues to emit beta radiation after the tumor fragments have been inoculated into mice.

Discussion

The primary purpose of this study was to compare the biological effectiveness of P^{32} radiation energy and that of x-rays, and to determine if the doses of radiation needed to achieve the effects under study are of the same order of magnitude in each case. A more quantitatively precise comparison would require a closer evaluation of the role played by the experimental conditions; that is, diffusion rate of phosphate ions into the soaking tissue, etc. It is very probable that when a tumor fragment has been soaked in radioactive phosphate solution it is subjected not to uniform irradiation, as in the case with x-rays, but rather the strength of the dose grades decreasingly from periphery to center of the tissue mass.

In Fig. 1 is also included the survival curve of mouse sarcoma 180 exposed *in vitro* to filtered 200 kv. roentgen rays (43). It shows that when mouse sarcoma 180 is irradiated *in vitro* the dose of filtered roentgen rays necessary to kill all the fragments of tumor is between 2,800 and 3,000 r (measured in air). On inspection, this would seem to indicate that in inhibiting the growth of tumor tissues, the energy released by P^{32} was found to be half as effective as x-rays under the condition of this experiment. However, this conclusion cannot be accepted as categorical because it is shown that tumor fragments immersed in the radioactive solution absorb a concentration of the isotope only about one-half that of the concentration in the surrounding fluid. Therefore, it could be argued that the total radiation effect on the tumor fragment was being produced by one-half the concentration of isotope used for the equivalent roentgen values upon which the curves in Fig. 1 are based; and that instead of the isotope energy being twice as biologically effective as x-rays, it is approximately the same.

Summary and Conclusions

1. An investigation has been made of the effect of immersing fragments of mouse sarcoma 180 in radioactive phosphorus solution prior to transplantation.

2. The growth capacity of mouse sarcoma 180 was unaffected when tumor fragments were immersed for

24 hours at 4-5° C. in P^{32} solution having an activity of 50 μc./cc. Immersion in P^{32} solution of 75 μc./cc. resulted in about 25 per cent inhibition. Marked inhibition and retardation of growth were caused by exposure to P^{32} solution of 100 and 125 μc./cc. (about 50 and 75 per cent inhibition, respectively). The viability of the mouse sarcoma 180 was completely destroyed by immersion in P^{32} solution of 150 μc./cc.

3. The lethal effect produced by beta rays of P^{32} on the tumor was compared with that produced by roentgen rays at 200 kv. since it is possible to calculate the beta-ray emission of the radioactive phosphorus in terms of equivalent roentgens. Under the stated conditions of the experiment it was found that the transplantability of mouse sarcoma 180 was not altered appreciably by irradiation of tumor fragments with 2,500 equivalent roentgens. An exposure of 3,500 equivalent roentgens gave about 20 per cent inhibition. Marked inhibition was caused by an exposure of 4,500 and 5,500 equivalent roentgens (about 50 and 80 per cent inhibition, respectively). The viability of the tumor was completely destroyed by an exposure of 6,500 equivalent roentgens.

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The Effect of Thorium Dioxide on Normal and Estrinized Tumor-Bearing Rats

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The effects of the disintegration products of thorium compounds have been extensively studied in man and animals (3, 4, 12, 18-21, 23). Thorium dioxide tends to disintegrate, over a long period of time, into mesothorium, radiothorium, thorium-X, and emanation, and its activity increases with age. This explains the appearance of many reports dealing with the effects of thorium dioxide or thorotrast many years after injections for diagnostic or experimental purposes (1, 7, 11).

Mora (9) reported a case of mammary tumor in a 40-year-old woman following injections of thorotrast into the breast. Selbie (16, 17) produced spindle cell sarcoma in 58 per cent of the rats and 26 per cent of the mice injected with thorium oxide and surviving for 12 to 18 months. He noted that, although the disintegration products of thorium dioxide are of low radioactivity, nevertheless they are sufficient, over a long period of time, to induce tumors in rats and mice. Foulds (2) has produced carcinoma and sarcoma in guinea pigs, while Roussy, Oberling, and Guérin (13-15), and Oberling and Guérin (10), have elicited sarcoma in rats. The period between injections of thorotrast and the appearance of tumors was from 12 to 18 months. Miyamoto (8) also has induced spindle cell sarcoma in rats, after 321 days. Homotransplants grew successfully.

These reports show that thorotrast requires a much longer time for the production of tumors than any other carcinogenic or radioactive substance. The delay has been explained by the fact that the radioactivity of degraded thorium dioxide *in vivo* is very weak. Taft (19, 20) has shown that 75 cc. of thorotrast C developed gamma radiation equivalent to 1.37 micrograms of radium.

Many published reports contain detailed descriptions of the general morphological and histological details of the tumors, varying from granuloma to spindle cell sarcoma, induced by thorotrast (2, 8, 10, 13-17).

No report dealing with the effect of thorotrast on spontaneous or transplanted benign tumors has been found in the literature.

Experimental Procedure

The purpose of the following experiments was to observe the effect of an injected radioactive substance in animals with spontaneous or transplanted benign mammary tumors.

Three female white rats with spontaneous mammary fibroadenoma and 24 with transplanted fibroadenoma were given 3 injections of 0.5 cc. of colloidal thorium dioxide.[1] Three rats with similar spontaneous tumors and 6 with transplanted tumors were given 1 mgm. of estrogenic hormone[2] in addition. Six normal and 6 estrinized rats without tumors were injected with like amounts of thorotrast to serve as controls. The results are summarized in Tables I and II.

It is well known that benign spontaneous and transplanted fibroadenomas in estrinized rats exhibit a marked hyperplasia of glands and ducts (5, 6) but our observations over a number of years have never shown a change from benign tumor to carcinoma in an injected rat. In an attempt to produce a malignant transformation in the benign growths of estrinized rats, thorotrast was therefore injected into, or in the vicinity of, spontaneous, autotransplanted, or homotransplanted tumors. All the animals were observed for the duration of life, and fragments of the spontaneous and transplanted tumors were removed at intervals for biopsy. At autopsy the viscera, lymph nodes, skeletal and muscular systems, and skin were sectioned and studied.

The average ages of the animals at injection and death and the periods of observation, with ranges shown in parentheses, are as follows: (a) for 6 rats with spontaneous tumors, age at injection 10.7 months (7 to 13), age at death 21.2 months (18 to 25), and period of observations 10.5 months (7 to 15); (b) for 19 rats with transplanted tumors, age at injection 8.9 months (2.5 to 15), age at death 15.6 months (10 to 25), and period of observation 6.7 months (4 to 14).

The growth rate and morphology of these benign spontaneous tumors in adult female rats were not influenced by the injected thorotrast, whether this was introduced into or near the tumors. Injections into the growth produced local areas of necrosis with contiguous hypervascularization. Autotransplanted fragments appeared to be mechanically inhibited by the inflammatory reaction produced by the bordering thorotrast. However, thorotrast injected at a site removed from the spontaneous tumor or autotransplant in no way stimulated or hindered growth. Similar observations

[1] Colloidal thorium dioxide or thorotrast (Heyden) is a stabilized aqueous solution, containing 25 per cent by volume of thorium dioxide (ThO_2), about 20 per cent by weight. The protective colloid is about 18 per cent by weight and is the dextrin fraction of a carbohydrate preparation. Thorotrast contains as a preservative 0.15 per cent of methyl p-hydroxy benzoate.

[2] The estrogenic hormone used in these experiments was Dimenformon benzoate, crystalline, synthetic, benzoic acid ester of alpha-estradiol (dihydroxy-estrin), generously furnished by Roche-Organon, Inc., through the courtesy of Dr. Louis Klein.

Ten thousand rat units represents 1.66 mgm. alpha-estradiol benzoate.

were recorded in the animals with transplanted fibroadenoma.

In rats receiving crystalline estrogenic hormone in addition, the glandular elements of the neoplasm were stimulated, as was to be expected, but the combined thorotrast and estrogen in no case produced any change in the morphology of the tumor pointing to malignancy.

In all the animals with spontaneous tumors, and in 66 per cent of the animals with transplanted growths, various types of swelling and induration developed at the site of the injected thorotrast. The transplanted fibroadenoma is elastic, soft, nodular, and purple, on gross appearance, while the growth induced by the thorotrast is hard, smooth, and white. The morphological characteristics vary from granuloma to spindle cell sarcoma. No metastases were seen, but infiltration of skeletal muscles in some rats and smooth muscle of the intestine in others by the thorotrast-laden cells was evident.

Homotransplants from one sarcoma induced by thorotrast grew in 10 (83 per cent) rats. In 6 rats

TABLE I: FEMALE WHITE RATS WITH SPONTANEOUS AND TRANSPLANTED MAMMARY FIBROADENOMA INJECTED WITH 1.5 CC. THOROTRAST

Rat number	Age when injected, in months	Age at death, in months	Spontaneous fibroadenoma	Transplanted fibroadenoma	Induced growth
1	12	21	+ *	+ *	Fibroma
2	12	22	+ *	+ *	Granuloma
3	12	19	+ *	+ *	Granuloma
4	12	21 †	—	+ *	Fibroma
5	12	14	—	+ *	Abscess absorbed
6	12	21 †	—	+ *	Granuloma
7	2.5	10 †	—	—	Abscess
8	2.5	10 †	—	—	Abscess
9	2.5	10 †	—	+ *	Abscess
10	15	24	—	—	Granuloma
11	15	22	—	—	Granuloma
12	15	24	—	+ *	Granuloma
13	15	19	—	—	Sarcoma
14	15	18	—	+ *	Abscess
15	15	?	—	—	Abscess
16	6	12	—	+ *	Granuloma
17	6	10	—	—	Granuloma
18	6	13	—	—	Sarcoma
19	6	12 †	—	—	Abscess
20	6	?	—	—	Abscess
21	6	13	—	—	Abscess
22	3	10	—	+ *	Abscess
23	3	17	—	+ *	Granuloma
24	3	14	—	+ *	Granuloma
25	3	16	—	+ *	Granuloma
26	3	10	—	+ *	Granuloma
27	3	14	—	+ *	Granuloma

* Morphology of spontaneous and transplanted tumors unchanged.
† Killed.

TABLE II: FEMALE WHITE RATS WITH SPONTANEOUS AND TRANSPLANTED MAMMARY FIBROADENOMA INJECTED WITH 1.5 CC. THOROTRAST AND 1 MGM. ESTRADIOL BENZOATE

Rat number	Age when injected, in months	Age at death, in months	Spontaneous mammary tumor	Transplanted mammary tumor	Induced tumor
1	7	18	Adenoma *	Adenoma * (autotransplant)	Granuloma
2	13	25	Fibroadenoma *	Fibroadenoma * (autotransplant)	Sarcoma
3	8	23 †	Fibroadenoma *	.....	Sarcoma
4	12	20	.....	Fibroadenoma *	Abscess
5	12	18 †	.....	.....	Granuloma
6	12	20	.....	.....	Abscess
7	2.5	10 †	.....	.....	Abscess
8	2.5	10 †	.....	.....	Abscess
9	2.5	10 †	.....	.....	Abscess

* Morphology of spontaneous and transplanted tumors unchanged.
† Killed.

transplants of fibroadenoma and thorotrast-induced sarcoma injected simultaneously grew independently, without affecting each other. In several of the animals the specific effects of thorotrast and estrogen could be noted in juxtaposition to the hyperplastic mammary glands from estrogen, sarcoma induced by thorotrast, and benign fibroadenoma growing from the transplant.

There was a notable increase of thorotrast-laden reticulum cells in the liver sinuses, spleen, and lymph nodes, conforming with the reports of many other investigators.

Discussion

With the exception of a carcinoma in one guinea pig described by Foulds (2) there is no record of a malignant epithelial growth induced in animals by thorium. Uehlinger and Schürch (22) conclude that in the rabbit, at least, radioactive agents act mainly on connective tissue and only rarely on epithelium. The recorded clinical observations also deal mainly with sarcoma following stimulation by radioactive agents, the only malignant epithelial neoplasms reported having been skin cancers.

Since the combined action of estrogen and a radioactive substance failed to induce the malignant change in benign epithelial tumors, other and still unknown factors must be postulated to explain the simultaneous occurrence of benign and malignant fractions in new growths.

Conclusions

1. Thorium dioxide did not produce malignant epithelial changes in benign fibroadenoma of the rat's breast.

2. The combined action of thorotrast and estrogenic substance did not induce a malignant change in either spontaneous or transplanted benign epithelial tumors.

3. The combined action of thorotrast and estrogenic substance did not elicit malignant epithelial tumors in normal rats.

4. Granuloma and sarcoma, however, could be induced by thorotrast whether or not the rat bore a spontaneous or a transplanted benign fibroadenoma.

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Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture

I. The Effect of X-Rays on Cell Outgrowth in Cultures of Normal Rat Fibroblasts and Rat Benzpyrene-Induced Sarcoma

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Exact knowledge of the direct effect of radium and x-rays on malignant cells is a primary condition for an understanding of the mechanism of the therapeutic action of radiation on neoplastic growth.

The investigations of the direct effect of radium and x-rays on living cells and tissues is greatly facilitated by the method of tissue culture. Normal and malignant cells of various types isolated from the body and kept in pure culture *in vitro* can be directly exposed to radiation and their response can be observed exactly and determined quantitatively. Moreover, by modifying the composition of the medium and other environmental conditions, the physiological state of the cell colonies may be varied, and the effect of these variations on the radiosensitivity of the cells can be investigated. In this way exact study of the direct effect of radiation on normal and malignant cells in different physiological conditions is made easy and comparison of the respective responses of normal and malignant cells to radiation is rendered possible.

The literature contains but few comparative studies of the radiosensitivities of malignant and normal cells *in vitro*.

Canti (1, 2) irradiated cultures of chick fibroblasts derived from normal periosteum and cultures of Jensen rat sarcoma with tubes containing 70 to 150 millicuries of radium emanation and found some differences in sensitivity to radium rays between normal fibroblasts and malignant spindle cells. Fibroblasts of normal periosteum showed very little change as a result of exposure to the emanation. The majority maintained their shape, their sharp outline, and the appearance of the nucleus was not altered. In the case of all the wandering cells, however, a marked effect was noticeable after 20 minutes' exposure; cell movement ceased and the cells became spherical with blurred, irregular outlines. When the cells of Jensen rat sarcoma were subjected to irradiation, apparently all of them, whether of the mobile or fibroblastic type, were affected in about the same time as the wandering cells of the normal tissue.

Laser and Halberstaedter (4) compared the growth-inhibiting action of radium rays on cultures of normal and malignant cells, and found that radium, in doses of 35 to 50 mgm. hr. which strongly reduced the growth of colonies of normal chicken osteoblasts, was practically without effect on cultures of Ehrlich mouse carcinoma and Jensen rat sarcoma. Doses of 12.5 mgm. hr. strongly inhibited the outgrowth of rat fibroblasts but had no effect on rat sarcoma cells (Crocker rat sarcoma 10).

Whitman (8) used cultures of Walker rat sarcoma for his experiments and compared the relative effect of 5, 16, and 50 mc. hr. doses of radium on the mitotic activity of malignant cells and normal macrophages always present in his tumor cultures. The results supported the conclusion that normal cells are more sensitive to irradiation than are malignant cells. "The number of mitoses of the normal cells (macrophages) was proportionately more reduced by irradiation than that of the malignant cells. The percentage initial fall in the mitotic count of the normal cells was greater for all three doses than for the malignant cells."

Goldfeder (3), Vollmar (5), and Vollmar and Rajewsky (6, 7) irradiated cultures of various tissues and tumors with x-rays of different dosage and intensity and described the effect obtained.

In view of the sparsity of these investigations and their conflicting results it seemed advisable to undertake a systematic study on the relative radiosensitivity of normal and malignant cells *in vitro*.

These investigations will be carried out on different experimental tumors, both mesenchymal and epithelial,

DESCRIPTION OF FIGURES 1 TO 6

PHOTOMICROGRAPHS OF UNSTAINED TISSUE CULTURES MAG. × 45

FIG. 1.—Rat sarcoma, culture No. 18723, nonirradiated.

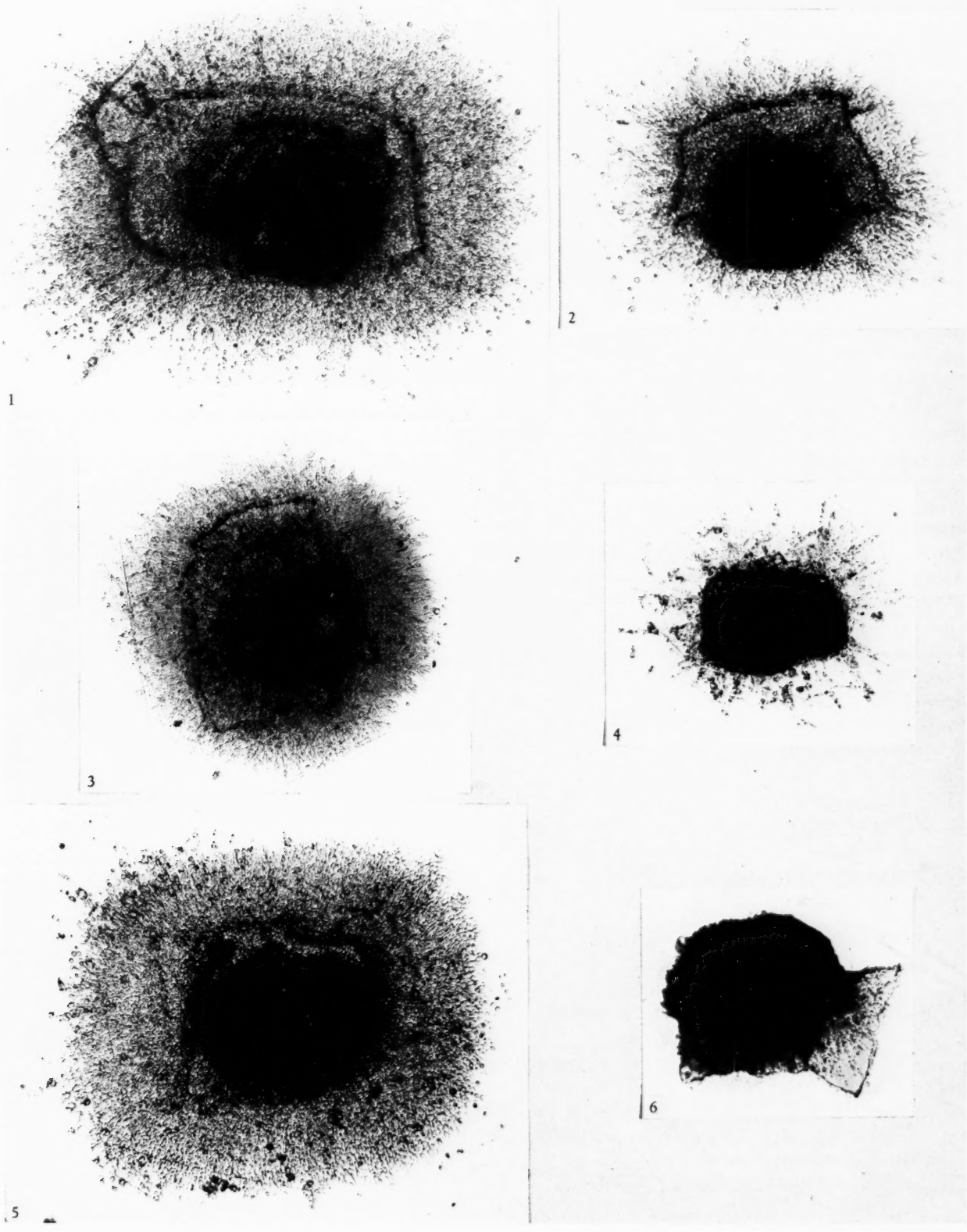
FIG. 2.—Rat sarcoma, culture No. 18274, from same source as Fig. 1, irradiated with 25,000 r. This shows + effect, continuous growth.

FIG. 3.—Rat sarcoma, culture No. 18370, nonirradiated.

FIG. 4.—Rat sarcoma, culture No. 18371, from same source as Fig. 3, irradiated with 25,000 r. This shows ± effect, slight growth.

FIG. 5.—Rat sarcoma, culture No. 18737, nonirradiated.

FIG. 6.—Rat sarcoma, culture No. 18738, from same source as Fig. 5, irradiated with 25,000 r. This shows — effect, no growth.



FIGS. 1 TO 6

and will comprise systematic studies of the radiosensitivity of the various vital functions of normal and malignant cells. The plan will be to compare the effect of radiation on the tumor cells with that on normal cells of the same species and type of tissue.

This first report is concerned with the comparative effect of x-rays on the outgrowth of normal rat fibroblasts and cells of rat benzpyrene-induced sarcoma in cultures.

Material and Methods

Cell cultures.—The experiments were performed on cultures of rat fibroblasts and on benzpyrene-induced rat sarcoma. The cultures of rat fibroblasts were obtained from explants of the extremities of approximately 10-day-old rat embryos. Cultures used in the irradiation experiments were 2 or 3 passages old.[1] The strain of sarcoma cells used in these experiments derives from a benzpyrene-induced rat sarcoma and has now been kept in this laboratory without loss of malignancy for one year. The cultures were made according to the standard cover slip method of Carrel. As culture medium we used normal chicken plasma and diluted chicken embryo extract in the proportion 1:1.

Irradiations.—Irradiations were carried out by means of a demountable x-ray tube operated at a tension of 35 kv. on a current of 20 ma. using copper anticathode. The window consisted of aluminium foil 30 μ in thickness. Absorption analysis showed that the rays which penetrated through the window foil and through the 0.03 mm. thick mica cover glass of the culture were mainly copper-k-rays. The x-ray intensity at the distance of the irradiated object was about 90,000 r/min.

For irradiation experiments fragments of the cell cultures were transferred into fresh medium, and irradiated immediately afterwards. After irradiation the cultures were put into the incubator at 37° C. The growth of the cell colonies was examined after 24 and 48 hours.

Experimental

The results of the experiments on rat sarcoma cultures have been summarized in Table I.

At a dose of 10,000 r all irradiated cultures developed continuous growth areas; their extension was always less than that of the outgrowth of nonirradiated cultures. At a dose level of 25,000 r, about one-half of irradiated cultures showed a continuous growth area. The remaining colonies mostly showed only isolated cells and a few failed to show any cell migration whatever. At 50,000 r the number of irradiated cultures with continuous growth area was negligible; about one-half of the cultures showed only single cells; in the remainder no cell migration whatsoever was observed. An irradiation dose of 75,000 to 100,000 r caused complete suppression of growth in the large majority of irradiated cultures. At 150,000 r only a few cultures which still showed some solitary cells remained. At a dose level of 200,000 r all outgrowth ceased.

Table I: Results of Irradiation of Cultures of Rat Sarcoma

Doses in r	Number of cultures irradiated	Effect of irradiation *		
		Continuous growth +	Slight growth ±	No growth −
10,000	14	14	0	0
25,000	26	12	10	4
50,000	27	3	13	11
75,000	39	0	16	23
100,000	48	0	17	31
150,000	63	0	5	58
200,000	29	0	0	29

* The column marked + indicates the number of irradiated cultures which showed continuous growth areas; column marked ± shows the number of cultures which after irradiation showed only solitary cells; column marked − gives the number of irradiated cultures in which not a single cell had migrated. Figs. 2, 4, and 6 give typical pictures of the irradiation effects classed respectively as +, ±, and −.

Table II: Results of Irradiations of Cultures of Normal Rat Fibroblast

Doses in r	Number of cultures irradiated	Effect of irradiation *		
		Continuous growth +	Slight growth ±	No growth −
10,000	14	14	0	0
25,000	23	14	8	1
50,000	27	0	17	10
75,000	44	0	21	23
100,000	136	0	25	111
150,000	65	0	3	62
200,000	28	0	0	28

* Notations same as Table I.

Table II summarizes the results of experiments on rat fibroblasts.

At a dose level of 10,000 r all irradiated fibroblast cultures show continuous growth zones. At a dose level of 25,000 r continuous growth zones were found in only 60 per cent of the irradiated cultures, the remainder showing only isolated cells. At 50,000 r none of the irradiated cultures showed continuous outgrowth, about 60 per cent solitary cells, and the remainder no migration whatsoever. At 75,000 r the number of cultures with single cells only was further sharply reduced. At 200,000 r all cell migration was completely suppressed.

[1] We have as yet been unable to obtain a permanent strain of rat fibroblasts. In the course of passages, the growth of rat fibroblasts under our experimental conditions becomes ever sparser. Only young and hence vigorously growing rat fibroblast cultures were, therefore, used in these experiments.

The results of the experiments have been arranged graphically in Fig. 7.

The courses of the curves for normal and malignant cells are seen to be practically identical. The minimum

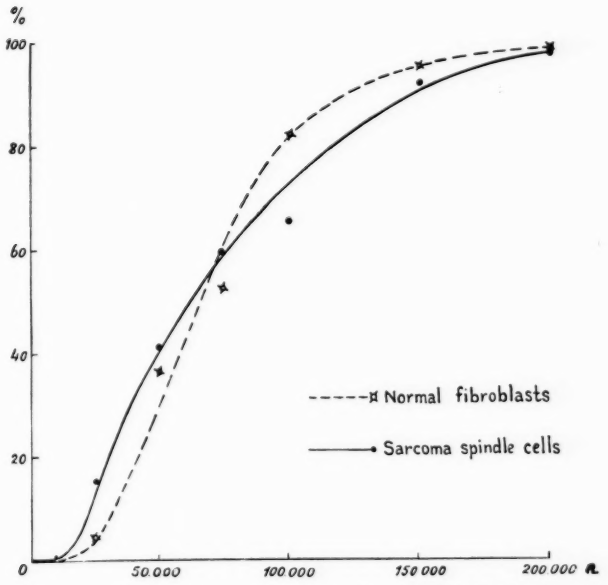


FIG. 7.—Graphic summary of results of the effects of x-rays on cell out-growth of normal rat fibroblasts and rat sarcoma in tissue cultures. The doses of irradiation in r are indicated on the abscissa. The percentage of cultures showing no signs of growth is shown on the ordinate.

x-ray dose which causes complete suppression of cell migration under our experimental conditions is the same for both normal rat fibroblasts and cells of rat sarcoma.

SUMMARY AND CONCLUSIONS

The minimum x-ray dose which totally suppresses cell outgrowth in tissue cultures is the same for both normal rat fibroblasts and cells of rat sarcoma. This dose is 200,000 r.

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The Behavior of Tumor Cells in Tissue Culture Subjected to Reduced Temperatures*

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The experiments with tissue cultures, described in this communication, were undertaken for a twofold purpose; namely, (a) to investigate the effect of different degrees of temperature upon the growth of both normal and neoplastic cells, and (b) to find the "critical" reduced temperature necessary to cause the death of cells of malignant tumors. The data from such studies, particularly those with the second objective, should be serviceable as a basis for comparative clinical studies of the application of reduced temperatures in the treatment of cancer in human beings. References to the more important historical and recent studies of the effects of hypothermy are included in the bibliography.

In 1938, Smith and Fay (26) reported the effect of both local and general hypothermy on tumor cells from a group of cases of advanced malignant disease, studied by means of serial biopsies. Regressive changes going on to complete tissue clearance in certain instances were observed. They advanced the theory that this resulted from a temperature differential for embryonal and neoplastic cells as compared with normal adult cells. Further evidence of the significance of the influence of temperature upon growth was presented by Smith (23) in recording developmental anomalies in chick embryos subjected to similar critical temperature levels during the first 72 hours of incubation.

On the other hand, it has long been known that tumor cells can be kept in the frozen state almost indefinitely at temperatures of $-70°$ C. or lower, without appreciable decrease of viability. This has been demonstrated repeatedly with transplantable animal tumor tissue both in bulk and by tissue culture methods (4, 8, 9-11, 21). The problem which presented itself was whether tumor cells could survive in the critical temperature range from 0° C. to 20° or 25° C. Our primary interest was in human neoplasms, but as a control, tissue from certain transplantable tumors of lower animals was likewise used.

Pertinent clinical observations, made simultaneously on the several tumors studied, will be commented upon briefly in connection with the tissue culture experiments.

* This investigation was aided by Grant No. 580 of the Committee on Scientific Research of the American Medical Association, and by a grant from the Clinical Research Foundation, Philadelphia, Pa.

Materials and Methods

Fragments of fresh, sterile, biopsied tumor tissue measuring approximately 7 mm. in diameter were kept at 0° C. for 12, 24, 48, and 60 hours respectively, to determine in each case the minimum lethal period. Several types of tumor were represented as follows: 1. reticulum cell sarcoma (lymph node), 2. colloid carcinoma (metastatic growth in abdominal wall from primary tumor of the sigmoid), 3. leukemia (acute lymphatic), 4. Hodgkin's disease (lymph node), and 5. mammary carcinoma of the mouse, (C57 Lankenau strain). All these tumors, including amelanotic malignant melanomas, were grown successfully by our tissue culture method (17, 18).

The use of tissue fragments of approximately uniform size contributed to the standardization of the technic. A diameter of about 7 mm. was selected after preliminary tests because fragments with diameters much less than 7 mm. were less resistant to refrigeration and fragments with diameters much larger than 7 mm. were likely to undergo degeneration centrally regardless of the temperature. These degenerative changes, caused probably by inadequate nourishment of the larger fragments, were apparently accompanied by the liberation of toxic metabolic products which exerted a deleterious influence on the otherwise vigorous tumor cells. This is a factor to be considered when attempts are made to cultivate cells from large, necrotic, degenerating tumors. No satisfactory explanation of the greater sensitivity of the smaller fragments to cold can be offered at this time.

The flasks used were those first described by us in 1930 (18). The initial medium used is what we call for convenience the 4, 4, 2 medium. It is made up of 4 drops of Tyrode's solution, 4 drops of heparinized chicken plasma, and 2 drops of 1:7 calf embryonic extract. The tissues were washed every 4 days with Tyrode's solution and a mixture of 4 drops Tyrode's solution, 2 drops of plasma, and 1 drop of extract added. The cultures were examined twice

daily, morning and evening. Interesting changes were noted and photomicrographs taken as indicated.

Following 48 hours' exposure at 0° C. before inoculation into the culture medium, all the tumors grew profusely at 37° C. The lag period which has been commented upon by Simms and Stillman (20) and others was found to be prolonged in direct ratio to the initial length of exposure of the tissue to these low temperatures. There was no evidence obtained to suggest that such tumor cells grew more rapidly or more profusely than those not submitted to this initial period of refrigeration. The rate of growth as indicated by the migration of the tumor cells from the inoculum varied considerably, especially in the leukemia and Hodgkin's disease tissue, but fibroblastic proliferation was well maintained. After 50 hours of preliminary exposure of the tissue at 0° C., growth was definitely affected, and many of the fragments of tumor tissue failed to grow. The lymphosarcoma and mouse carcinoma tissues showed greater resistance to the preliminary refrigeration, but rarely grew after 60 hours of exposure to 0° C.

The next point to be established experimentally was the temperature level which would slow down the rate of proliferation of tumor cells and thus perhaps lead to their ultimate destruction. Two methods were employed: in one, the tumor tissue was placed immediately in the culture medium at the desired low temperature; in the other, it was subjected first for a 24-hour period to 37° C. before the lower temperature was applied. This initial period at body temperature permitted the tumor to become better established so that its survival at the lower temperature level was somewhat prolonged. In other respects its behavior was entirely comparable to that of the cultures placed at once at the lower levels.

So far as possible fresh biopsied tissue was used for the tests at each experimental temperature level. However, in dealing with human tissues, this ideal plan was not always practical, and "stock" cultures and subcultures had to be used at times to complete any single series of observations. It was further found that tumor tissue taken at autopsy from bodies dead as long as two hours would grow equally satisfactorily provided there was no bacterial contamination present and the tissue could be removed aseptically. No attempt was made to grow the tumor cells in pure culture, except incidentally. We tried to duplicate *in vitro,* so far as possible, the same relationship in respect to the tumor cells and their supportive stroma as found *in vivo.*

Results

Reticulum cell sarcoma.—The tissue was obtained by biopsy from Cancer Research Case No. 67, a 2½-year-old boy on Dr. Temple Fay's service, who had a rapidly progressive reticulum cell type of lymphosarcoma of the mediastinum with extreme secondary involvement of the cervical lymph nodes. The masses in the neck showed marked regression under local application of cold (5° C.) but the patient died later as a result of the primary mediastinal tumor.

The biopsied material consisted of a lymph node which had undergone extensive softening and necrosis. Microscopic examination of the tissue showed definite degenerative changes of the remaining cells and it was felt doubtful whether any growth would be obtained by culture.

At 37° C., however, the tumor grew well; the fibroblasts first, followed by the reticulum cells. After 3 to 4 days, the fibroblasts were overgrown by the primary tumor cells and rapidly died off, leaving a practically pure culture of reticulum cells. These grew larger and larger until they were four or five times their original size, and showed the most bizarre pleomorphism. There was diffuse migration of the tumor cells through the medium to form a far-flung reticular network.

The culture was subjected to a temperature of 40° C. for 48 hours. No permanent injury to the tumor cells was noted, but there was a definite lag period in their growth for the first few days following this exposure.

Further evidence of the natural resistance of these tumor cells to adverse conditions was obtained in another experiment. For over 6 weeks the cultures were simply washed at weekly intervals and fresh buffered Tyrode's solution added. The colonies shrank in size, became granular, and as far as could be determined microscopically, the cells appeared nonviable. This was true not only of the material at 37° C. but also that grown at 26° C. At the end of the 6 weeks' period fresh embryo extract was obtained and after a lag period of about 10 days, growth was re-established.

Cultures grown at 26° C. either immediately, or following an initial 48 hours at 37° C., grew almost equally as well as controls at the normal body temperature, after an initial lag period during which the cells apparently became adapted to the lower temperature level. They were maintained for 6 months. Tissue in this case, obtained at autopsy some 2 hours after death, exhibited the same growth capacities as the tissue obtained by biopsy.

Colloid carcinoma of colon.—The tissue in this case (Case No. 62) was obtained by biopsy from a metastasis implanted in the abdominal wall, the primary tumor being of the descending colon with extensive intra-abdominal metastases producing partial obstruction. Under combined general and local lowered temperature, the minimum being 29° C., clinical improvement was noted with regression in the size of the

main tumor mass, disappearance of the obstructive symptoms, and marked relief of pain.

The tumor was treated in the same way in tissue culture; *i. e.,* grown at 37° C. immediately; grown at 37° C. after 48 hours' exposure to 0° C., with resultant congelation; grown at 26-27° C. immediately, and also following 48 hours at body temperature. The rate of growth in all instances was much less than that observed with the reticulum cell sarcoma, and the lag period was especially marked in the material congealed at 0° C. for the first 48 hours. The cells migrated, but extremely slowly in all cultures. In the 26-27° C. series there was pronounced degeneration of the cells, with karyolysis of the nucleus and rupture of the cell membrane resulting in a spilling of the cell contents into the medium. This could be followed readily under the microscope.

Leukemia.—Two cases of acute lymphatic leukemia and one of myeloid leukemia were studied to a limited extent. Little added information was gleaned, except that a drop in temperature of only 5 or 6° C. would halt the migration of the myeloblast. Unfortunately, lacking suitable motion picture equipment, we could not follow the various aspects of this characteristic migratory phenomenon as described by Rich, Wintrobe, and Lewis (16), and were unable to confirm the typical "angleworm" appearance of these cells in migration. On the other hand, the "hand mirror" form of the lymphoblast in cultures from lymphoid leukemic cells was frequently observed.

The cultural behavior of the lymphoid series of cells from cases of acute lymphatic leukemia is surprisingly constant, and they are among the easiest of tumor cells to cultivate. In tissue cultures made from fragments of biopsied lymph nodes from leukemic material the same lag period is noted as in the cultures of cells from other tumors if the tissue is subjected to preliminary congelation for 48 hours; but otherwise the sequence of events is the same. Within the first few hours there appears a halo of cells around the explant. This is assumed to be evidence of the liberation of the lymphocytes from the tissue during inoculation into the culture media.

Within 4 to 5 hours at 37° C. there is fibroblastic proliferation. Such fibroblasts appear unusually large and elongated. After 24 hours the small lymphocytes migrate and are followed by the reticulum cells. At about this period or shortly thereafter the lymphoblast makes its appearance and can be identified by its hand mirror (16) form during migration.

As the temperature is lowered the fibroblasts grow progressively smaller, less spindle-shaped, and more oval in outline. This can be readily observed around 30° C. and is very striking at 25° C. Similarly the reticulocyte and lymphocyte become less and less conspicuous as the temperature level is dropped. Proportionally the number of lymphoblasts is increased. When the culture is returned to 37° C., growth again becomes profuse with the fibroblast predominating. The adult lymphocyte has almost entirely disappeared and the lymphoblast is still in evidence. These changes are shown in Figs. 1 and 2.

The fluctuations in the total white count in the peripheral blood (Fig. 3) were especially noticeable in Case No. 96 (services of Dr. T. McNair Scott and Dr. Temple Fay) before, during, and after generalized hypothermy. The relative proportions of the adult and blast forms were remarkably similar to the proportions of these cells as observed in tissue cultures. These additional data are omitted from Fig. 3 for purposes of simplification as the essential feature to be observed is the sharp drop in cell count following each period of refrigeration.

Hodgkin's Disease.—Two cases of Hodgkin's disease (Temple University Hospital Nos. 29625 and 29821) were available for study.

In general, the cultural characteristics of the various component cells of biopsied lymph nodes from cases of Hodgkin's disease, with the exception of the Reed-Sternberg type of cell, were in every way similar to those observed in the tissues from the cases of lymphoid leukemia. However, in this and other cases of Hodgkin's disease which had been irradiated over a prolonged period of time with resultant fibrosis of the tissues, it was difficult to obtain any growth, even of the supportive stromal connective tissue cells.

The special point of interest in these cases of Hodgkin's disease is in respect to the Reed-Sternberg giant cells. Their characteristics in cultures have been described by Lewis (12). At 37° C. the typical multinucleated giant cells could be readily identified, but as the temperature level was lowered the behavior of other cells was studied especially.

At 24° C. large cells, twice the size of the normal histiocyte, and containing a large, ovoid, dense, single nucleus and fine cytoplasmic granules were seen. Under continuous observation the cell gradually increased in volume, so that the relative proportion of cytoplasm to nucleus was increased. Next the nucleus became swollen at one pole, with a zone of apparent constriction, until the original nucleus was surmounted by a sessile sphere, slightly smaller than itself, and still attached to the parent structure. There was no intimation of cytoplasmic involvement in the process. This newly formed nuclear cap then budded in turn, and the process was repeated until four small spheres were formed, attached to each other in chain fashion, each nuclear bud being somewhat smaller than the one which had given it birth. There was

FIG. 1.—Photomicrograph of a 7-day tissue culture from a lymph node from Hodgkin's disease (Temple University Hospital Case No. 29821) incubated at 37° C. Note profuse growth of large fibroblasts with typical spindle-shaped nuclei and slender parallel loose cytoplasmic processes. Toluidin blue stain. Mag. approx. × 400.

FIG. 2.—Photomicrograph of a 7-day tissue culture from the same origin as that shown in Fig. 1, incubated at 24° C. The fibroblasts are small, shorter, and stubbier, more compactly arranged and the nuclei are less spindle-shaped. Toluidin blue stain. Mag. approx. × 400.

no evidence of mitosis during this entire process which was completed in the course of 60 hours (Fig. 4).

Following this phenomenon, the cultures were placed in the incubator at 37° C. to see whether this abnormal nuclear division would be completed. In no instance was any further nuclear change observed although normal fibroblastic growth promptly reappeared, indicating that conditions for cell growth were satisfactory. This check or frustration of nuclear division was noted in several of the cultures, although not always so strikingly. The process was invariably one of budding.

Mammary carcinoma of mouse (C57 Lankenau strain).—With this material, which was available in almost unlimited amount, we were able to carry out a large number of experiments in our effort to find the temperature levels which interfered with, or actually stopped mitosis and killed the tumor cells.

It was found that the cell of the mouse carcinoma was able to resist exposure at 0° C. for as long as 40 hours just as human tissue could. When placed immediately at 37° C. the fibroblastic proliferation was not as profuse as in human tumor tissue, nor did it persist as long. In general, the behavior of the mouse carcinoma tissue was in all respects similar to that of human tissue through the whole temperature range from 25-37° C.

At 24° C. migration of the tumor cells lagged perceptibly and the number of mitoses was relatively increased. It was possible to follow some of these cells under the microscope. Many of them failed to go beyond the metaphase, some few reached the anaphase, and occasional cells even attained the telophase. The majority of these mitotic figures appeared normal and balanced, so far as they went; although now and then an unbalanced chromosomal division was noted.

The particular point of interest lay in the fact that at this temperature level (24° C.), provided the tissue was subjected to it for a period of 24 hours or longer, these mitoses never went on to completion even if the cultures were subsequently restored to 37° C. On the other hand those tumor cells not involved in this process proliferated normally again when back at body temperature.

The influence of temperature on mitosis in animal cells has been recognized for a long time (11, 21). The physical property of the cytoplasm of the cell during mitosis normally changes from a fluid mass to a jelly-like consistency. For mitosis to become completed refluidification must take place. Obviously during this process the fluid-gel balance is in very delicate equilibrium. It is not improbable that the lowered temperature upsets this equilibrium and thus arrests the completion of the cell division.

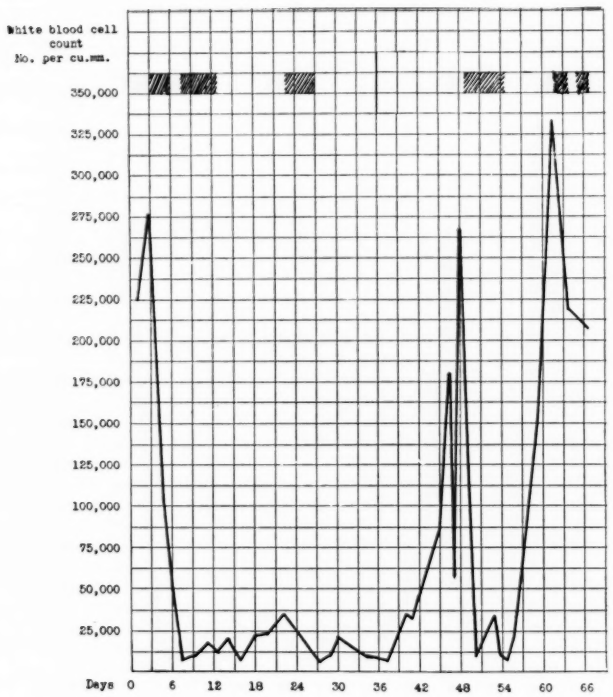

FIG. 3.—Total counts of white cells in peripheral blood in a case of acute lymphatic leukemia (Case No. 96) during a period of 68 days, showing fluctuations under treatment with general hypothermy. Note sharp drop in the count following each period of reduced temperature. Periods of reduced temperature are indicated by shaded areas across the top of the chart.

At 22° C. very little cell activity was noted, save for the occasional growth of a dwarfed fibroblast. Exposure at this temperature for as long as a week was not regularly lethal, but any considerable further prolongation of the time interval was usually sufficient to prevent growth when the cultures were placed again at 37° C.

At 20° C. shorter time intervals were found to be lethal; 5 days was the lower limit in all instances. It is difficult to determine accurately at which temperature *all* tumor cells are destroyed. There is no question that their viability varies considerably. If fifty colonies from the same tumor are planted and treated as nearly identically as possible, one or two of them invariably will be found to survive exposures to low temperatures which will be lethal for the others. Statistically, this is of comparatively little importance, but in terms of human disease, it becomes an almost insurmountable problem, for a single, viable, unscathed tumor cell could, theoretically at least, perpetuate the neoplastic process.

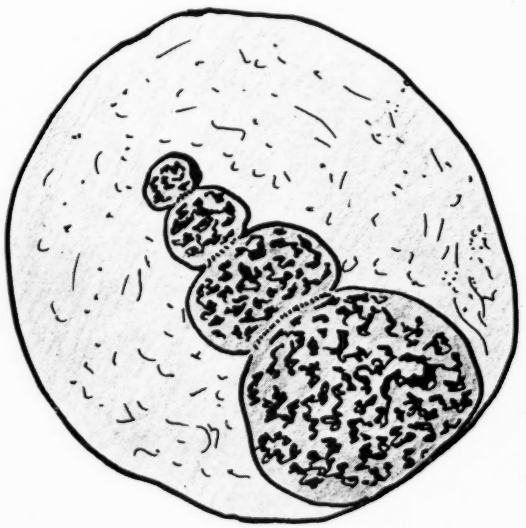

FIG. 4.—Diagrammatic sketch of a giant cell in a tissue culture from a lymph node from Hodgkin's disease showing "checked" mitosis as the result of incubation at reduced temperature.

Control material.—Hundreds of cultures of normal human fibroblasts were subjected to the same experimental procedures. Lowering the temperature caused a definite slowing of the rate of growth, but nowhere nearly as marked as in the case of the tumor cells studied. Conversely the recuperative and regenerative capacities of the fibroblast were found to be greater. These studies will be published separately.

Normal lymph nodes from the mouse were studied in tissue culture in a similar fashion. As in the case of the fibroblast, migration of the normal adult lymphocyte was much less prompt. In the 24-37° C. range a delay of 4 to 6 hours was regularly noted as compared to the migration of the neoplastic lymphoblast. Likewise, demonstrable degenerative changes were delayed by as much as 18 to 24 hours and required a much longer period of time for their completion in comparison with the lymphoid cells grown from leukemic nodes.

TABLE I: SUMMARY OF EXPERIMENTAL AND CLINICAL OBSERVATIONS

OBSERVATIONS *IN VIVO*						OBSERVATIONS *IN VITRO*			
Case	Clinical diagnosis	Dates	Amount and type of hypothermy	Applied temperature	Clinical effects	Dates	Applied temperature	Effects in tissue culture	Number of cultures
C. R. P.* No. 67, 17 mos. ♂	Reticulum cell sarcoma of anterior mediastinum with cervical node metastases	10/30/39	24 hrs., general	30° C.	Slight regression (as shown by x-ray)	10/30/39	37° C.	Rapid growth in 24 hours	50
						11/3 to	24° C.	Marked shrinkage in size of colonies and of individual cells	
		11/3/39	24 hrs., general	30° C.	Further regression in size of mediastinal mass	11/10/39			
						11/10 to	30° C.	Marked granular shrinking and apparent loss of viability during prolonged periods of inadequate nutrition	
		10/30 to 11/14/39	15 days, local to glands of neck	5° C.	Marked local decrease in size of glands	1/10/40			
						1/10/40	37° C.	New media—resumption of normal growth	
						1/10 to 6/10/40		Normal growth over 6-month period. Discarded	
C. R. P.* No. 91, 3½ yrs.	Acute lymphatic leukemia	12/5/39	25 hrs., general	30° C.	Decrease in number of lymphocytes	12/5/39	37° C.	Rapid migration of lymphocytes	30
						12/6/39	24° C.	Rapid degeneration of lymphocytes after 24 hours	
C. R. P.* No. 96, 3½ yrs. ♂	Acute lymphatic leukemia	12/27/39	None	37° C.	WBC per cu. mm.—270,000	12/29/39	37° C.	Rapid migration of lymphocytes and lymphoblasts	50
		1/1/40	72 hrs., general	30° C.	WBC per cu. mm.— 40,000				
		1/3/40	110 hrs., general	30° C.	WBC per cu. mm.— 10,000	12/30/39	24° C.	Degeneration of lymphocytes	
		1/8 to 1/17/40	None	37° C.	WBC per cu. mm.— 33,500				
		1/22/40	90 hrs., general	30° C.	WBC per cu. mm.— 13,450				
		1/26/40	120 hrs., general	30° C.	WBC per cu. mm.— 10,400	2/3/40	37° C.	Profuse growth of fibroblasts and migration of lymphocytes	
		1/26 to 2/10/40	None	37° C.	WBC per cu. mm.—173,000				
						2/4/40	30° C.	Degenerative changes in lymphocytes after 24 hours	
	288 hours local hypothermy 5-10° C. to glands of neck and groin	2/12/40	48 hrs., general	26° to 30°	WBC per cu. mm.— 66,150				
		2/16/40	120 hrs., general	"	WBC per cu. mm.— 21,500	2/23/40	37° C.	Good growth and migration of lymphocytes	70
		2/16 to 2/23/40	No general	37° C.	WBC per cu. mm.—136,000				
						2/24/40	24° C.	Rapid degeneration of lymphocytes	
		2/27/40	No general	37° C.	WBC per cu. mm.—334,000				
		2/29/40	48 hrs., general	30° C.	WBC cu. mm. per—214,000				
C. R. P.* No. 62, 55 yrs. ♂	Colloid carcinoma of transverse colon	10/28/40	24 hrs., general	30° C.	Disappearance of edema	10/23/40	37° C.	Very slow growth	50
		11/2/40	72 hrs., general	27° C. 30° C.	Total relief of intestinal obstruction	10/28/40	24° C.	Degeneration of tumor cells	
		11/11/40	96 hrs., general 102 hrs., local	27° to 30 °C.	General improvement—local regression	11/11/40	37° C.	Very poor growth	50
		11/21/40	72 hrs., general	"	Relapse				
		12/6/40	80 hrs., general	"	General improvement				
		12/27/40	80 hrs., general	"	Discharged improved—died at home one month later				
T. U. H.† No. 29625, 27 yrs. ♂	Hodgkin's disease	9/23/40	Treated by x-ray		Regression	9/23/40	37° C. 24° C. 60 hrs.	Good growth Shows growth of fibroblasts, migration of lymphocytes and giant cells, some of which show "checked" division	70
						9/26/40	37° C. 24° C.	Same as 9/23/40	
						9/28/40	37° C. 24° C.	Same as 9/23/40	
T. U. H.† No. 29821, 17 yrs. ♂	Hodgkin's disease and tuberculosis	10/17/40	Treated by x-ray		Regression	10/17/40	37° C. 24° C.	Poor growth Giant cells of unspecified identity—quite evident	70
Breast carcinoma in Lankenau C57 strain of mice		12/5/40 1/7/41	Mice not treated			12/5/40	37° C. 24° C.	Rapid growth after 6 hours Growth less than at 37° C. Mitoses more numerous. Checked mitoses after application	300

* Cancer Research patient. † Temple University Hospital.

Discussion

As a result of these experiments at varying temperatures it becomes apparent that there is a definitely "critical" level around 22-24° C. which, if maintained over an adequate period of time, results in lethal interference with the metabolism of neoplastic cells in tissue cultures. Such a low body temperature has been attained at least once in a case of human cancer (25), and would seem to be within the realm of further practical investigation clinically. Obviously, its application at this stage of knowledge would be in cases of inoperable malignant tumors which had also received the maximum irradiation possible, especially in cases with deep-seated metastatic lesions.

Previous studies have thoroughly established the value of local hypothermy at temperatures of about 4-5° C. in accessible tumors (6, 7, 26). The earlier experimental observations of general hypothermy suggested that at 30° C. marked regressive changes in tumor tissue followed quite regularly (24). With the evidence which these tissue culture experiments supply in respect to the cytoplasmic and nuclear changes of tumor cells at a slightly lower level, 22-24° C., we feel that the vulnerability of the tumor to injurious agents is increased. If, as is generally believed, cells are more susceptible to x-ray during the period of mitosis, then it would seem logical, by inducing by hypothermy a state of checked mitosis or arrested cell division, that irradiation might well be more effective at such a period. Subsequent investigation should be directed to working out an optimal combined therapy. It does not seem unlikely that the x-ray dosage might be reduced under such a program, thus making it possible to prolong the effective period of therapy without causing severe damage to normal tissues.

Summary

1. The behavior of tumor tissue from cases of reticulum cell sarcoma, colloid carcinoma, acute lymphoid leukemia, Hodgkin's disease, and mammary carcinoma of the mouse (Lankenau C57 strain) under varying temperatures ranging from 0-37° C. has been studied *in vitro*.

2. The "critical" level of 22-24° C. has been shown to be of particular significance in respect to nuclear division.

3. The importance of the factors of actual temperature, time, and individual cell resistance have been emphasized.

4. A parallel has been drawn between the tumor response to hypothermy *in vitro* and *in vivo*.

5. A discussion of the practical clinical application of the principles established by these studies in regard to human cancer is presented.

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The Relationship of the Endocrine System to Carcinogenesis*

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Since several members of the endocrine system are known to influence metabolism, and other hormones are concerned in both genital and somatic growth, the abnormalities of metabolism and growth which are characteristic of tumor development might conceivably be due to, or at least influenced by, derangements in the supply of hormones normally governing such processes. A number of studies concerning one or another of the endocrine glands in relation to spontaneous, transplanted, or chemically induced tumors have been published. However, conclusions based on a comparison of results when tumors of different origin are under consideration are probably not valid. The wide variations in susceptibility between strains of animals used by different investigators must be taken into account. In the present study, the influence of each of the endocrines upon tumors having the same origin was determined in one strain of rats. As this study concerns only chemically induced tumors, no attempt will be made to review the literature covering endocrine influences upon spontaneous and transplanted tumors. References dealing with such relationships to chemically induced tumors will be cited under the appropriate sections.

Materials and Methods

The plan adopted was to investigate separately each member of the endocrine system, both as regards under-supply and over-supply of its secretion. As this work was primarily to serve as a base for further study, no combination of hormones was used. The state of under-supply was produced by extirpation of the gland involved; the condition of over-supply by administration of the appropriate hormone, usually in the form of a commercial preparation,[1] whose biologic activity was checked by us.

Carcinogen used.—Methylcholanthrene was employed throughout, with the exception of one group in which 1,2,5,6-dibenzanthracene was used. The primary reason for the choice of methylcholanthrene was the fact that in our strain of rats we obtain 100 per cent tumor production with a 1 per cent solution of this agent in paraffin. This is an advantage when compared with the uncontrollable variability of occurrence of spontaneous tumors and the uncertainty of transplants' taking. The comparatively short latent period with methylcholanthrene is also advantageous. Dibenzanthracene has a much longer latent period; it was used in one of the hyper-estrin groups because it was felt that the suspected stimulating power of estrin on tumor growth might be more apparent in the case of a more slowly acting carcinogen.

Administration of the carcinogenic agent.—The carcinogens were administered by the subcutaneous route. A 1 per cent solution was prepared in paraffin of low melting point and a total of 1 cc. injected into each animal at 5 different sites, 2 mgm. thus being placed at each point. One injection was made over the shoulders, one over each hip, and one in the mammary line at either side of the abdomen. Exact duplication of these points was attempted in each animal injected.

Animals used.—Young rats 6 weeks old at the time of injection were used. The animals were of the Denver strain, developed through 10 years of inbreeding and having nearly a zero incidence of spontaneous tumors.

Measurement of tumors.—The presence of growing tumors was determined by simple palpation. The size adopted as showing termination of the latent period; that is, the time elapsing between injection of the carcinogen and the formation of a growing tumor, was a mass of 1 cc. Some difficulty was experienced in the accurate measurement of certain tumor masses, as occasionally they have a tendency to grow as flat discs, even after active growth is in process. In these cases, allowance was made for thinness by greater surface area, so that a total of 1 cc. mass was present before it was counted as an active tumor. In all cases, several individuals frequently checked the tumors independently and agreement was

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[1] Thanks are expressed to the following companies for their generous contribution of hormone preparations: Armour and Co., G. W. Carnrick Co., Ciba Pharmaceutical Products, Inc., Cudahy Packing Co., Cutter Laboratories, Lakeside Laboratories, Inc., Parke, Davis & Co., Schieffelin & Co., the Schering Corporation, E. R. Squibb & Sons, The Upjohn Co., Wilson Laboratories, and the Winthrop Chemical Co., Inc.

reached before the tumor was considered to have the required volume.

Experimental groups.—1. Gonadal hormones. a. Hyper-estrin. The estrogen used was estradiol benzoate (progynon-B, Schering Corporation). A noncancerous group was injected as controls with the same doses of estrogen, no tumors resulting. The work of many investigators has led to the belief that estrogens may be concerned in some types of tumors. This literature has recently been reviewed by Gardner (4).

b. Hyper-progestin. This group was injected with progestin (Parke, Davis & Co., Lakeside Laboratories, Inc.) in oil, from crystalline preparations. A noncancerous group was injected with the same amount of progestin, with no tumors resulting.

c. Hypo-estrin. The animals were ovariectomized 10 days previous to the administration of the carcinogen. Boyland and Warren (1) have obtained what they believe to be a significant difference in the percentage of tumors induced with methylcholanthrene in ovariectomized mice.

d. Hyper-testosterone. Testosterone propionate (perandren, Ciba Pharmaceutical Products, Inc., and oretone, Schering Corporation) was administered at two dosage levels. Noncancerous groups were given equivalent amounts, with no tumors resulting. Lacassagne and Raynaud (7) found testosterone to inhibit mammary tumor development. Flaks and Ber (3) found that testosterone acts to delay formation of methylcholanthrene-induced tumors in mice.

e. Hypo-testosterone. Castration was performed 10 days before injection with the carcinogen. Stewart (9) has been unable to reach a definite conclusion on the effects of castration in the development of 1,2,5,6-dibenzanthracene tumors.

2. Gonadotropic hormones. a. Pregnant mare serum. Two groups of rats were used, one on a high, the other on a low dose. The material used was gonadin (Cutter Laboratories).

b. Pregnancy urine. Again two dosages were given; the preparation was korotrin (Winthrop Chemical Co., Inc.).

3. Adrenal hormones. a. Hyper-cortin. The material used was: adrenal cortex extract (Wilson Laboratories), eschatin (Parke, Davis & Co.), cortalex (The Upjohn Co.), and cortisorbate (Schieffelin & Co.) Because of the large amounts necessary in an experiment of such long duration, only 5 rats were used. The hormone was given partly orally, in the form of a charcoal adsorbate mixed with the diet, and partly subcutaneously.

b. Hyper-desoxycorticosterone. The material used was percorten (Ciba Pharmaceutical Products, Inc.) Again only 5 rats were used.

c. Hypo-cortin. The adrenals were removed one week after the injection of the carcinogen. A maintaining dose of cortin was supplied in minimal amounts, one-half r.u. daily in the diet, and supplemented, when necessary to maintain life, by injection of cortical extract.

d. Hyper-adrenalin. The hyper-state was obtained by subcutaneous injection of adrenalin in oil (Parke, Davis & Co.), twice daily, in an effort to maintain a nearly constant action of the hormone. Goetsch (5) has found that adrenalin at this concentration will act for about 12 hours.

e. Hypo-adrenalin. The medulla of each adrenal was removed as completely as possible through a slit in the adrenal cortex.

4. Thyroid and pancreas. a. Hyper-thyroid. An extreme degree of hyperthyroidism was maintained by the injection of thyroxyl (E. R. Squibb & Sons). When the toxicosis threatened the survival of the animals, injections were stopped and smaller amounts administered in the diet for a few days.

b. Hypo-thyroid. Thyroidectomy was performed a week after injection of the carcinogen. Every effort was made to remove the entire gland, weights were recorded daily, and any fair gains in weight disqualified the animal from further consideration. No further injections were given, although calcium lactate was given in the water for a few days after the operation.

c. Hyper-insulin. Protamine zinc insulin (E. R. Squibb & Sons) was employed because of its prolonged action. Much higher doses than of ordinary insulin were tolerated. Because of the long duration of the experiment, the animals could not be starved. Blood sugar determinations were made at frequent intervals, and a fluctuating, but continuous, condition of hypoglycemia maintained.

d. Hypo-insulin. The pancreas was removed as completely as possible, using the method of Richter and Schmidt (8), one week following administration of the carcinogen. Determinations of glucose in the urine were made frequently during the development of the tumors.

5. Pituitary hormones. a. Hyper-pituitary. A crude pituitary extract was prepared from fresh sheep pituitaries, using the method of Van Dyke and Lawrence (11). Five mgm. of the precipitate was equivalent to 1 gm. of fresh tissue. The preparation was given in saline soluton made up daily.

b. Hyper-growth hormone. The preparation used was phyone (Armour and Co.) of which 1 cc. contains the activity of 0.133 gm. of fresh anterior lobe tissue. Weight records were kept of each rat.

c. Hyper-prolactin. International standard prolactin was used in this group, the preparation being made up daily.

d. Hypo-pituitary. The pituitaries were removed by a modification of the method of Thompson (10). No serial sections were made at autopsy because, if cessation in weight gain and failure of gonadal development became apparent, it was felt that the hypo-pituitary state had been reached. Any animals showing normal development were disqualified. Korteweg and Thomas (6) have shown, as have others, that removal of the pituitary results in some inhibition of tumor formation in mice.

6. Controls.—Several lots of methylcholanthrene were used during the course of this experiment and a number of control rats injected from each lot. No significant difference in the length of the latent period in these groups was observed. Especial care was exercised in the checking and measuring of these groups to insure reliable control data. A second group of controls consisted of animals injected with paraffin only. These were followed for a period of one year; no tumors of any sort developed.

Results

The results are summarized in Tables I and II.

Discussion

This work represents an attempt to influence the rate of development of chemically induced tumors by

Table I: Methylcholanthrene

Group	Number and sex of rats	Hormone dose per week	Injection schedule	Mean latent period, in days	σ	Critical ratio $\left(\frac{D_m}{\sigma_D}\right)$
Hyper-estrin	5 M	30 r.u.*	3 × weekly	111.0	15.36	0.59
Hyper-estrin	5 F	30 r.u.	3 × weekly	105-1	14.96	0.24
Hyper-estrin	5 M	750 r.u.	3 × weekly	119.0	34.10	2.06
Hyper-estrin	5 F	750 r.u.	3 × weekly	109.6	20.61	0.41
Hypo-estrin	10 F	.....	.....	104.2	10.00	0.59
Hyper-progestin	10 M	3 mgm.	3 × weekly	107.5	14.53	0.14
Pregnant mare serum	10 M	1.5 r.u.†	3 × weekly	89.2	18.92	2.73
Pregnant mare serum	10 M	15.0 r.u.	3 × weekly	79.1	9.04	7.10
Pregnancy urine	10 M	1.5 I.U.	3 × weekly	87.8	19.41	2.50
Pregnancy urine	10 M	15.0 I.U.	3 × weekly	87.4	19.81	2.40
Hyper-testosterone	10 M	60 gamma	3 × weekly	100.9	18.74	0.83
Hyper-testosterone	10 M	600 gamma	3 × weekly	109.1	19.17	0.35
Hypo-testosterone	10 M	.....	.....	107.2	19.23	0.58
Hyper-cortin	5 M	23 r.u.‡	Daily	122.5	15.30	2.10
Hyper-desoxycorticosterone	5 M	3 mgm.	3 × weekly	97.8	11.70	1.57
Hypo-cortin	5 M	.....§	.....	106.6	12.60	0.06
Hyper-adrenalin	10 M	3.5 cc. 1: 10,000	2 × daily	97.3	17.77	1.76
Hypo-adrenalin	8 M	.....	.....	103.5	7.63	0.87
Hyper-thyroid	31 M	25 mgm./kg.	Varied‖	106.5	19.68	0.07
Hypo-thyroid	14 M	.....	.....	108.9	17.43	0.40
Hyper-insulin	12 M	48 U./kg.	Varied¶	105.4	17.12	0.33
Hypo-insulin	10 M	.....	.....	109.7	16.09	0.52
Hyper-pituitary	10 M	2 gm. fresh tissue	Daily	114.0	19.5	1.00
Hyper-growth	10 M	0.2 gm. fresh tissue	Daily	97.9	15.9	1.60
Hyper-prolactin	10 M	10 I.U.	3 × weekly	111.8	10.69	1.25
Hypo-pituitary	8 M	.....	.....	112.0	11.13	1.10
Controls	50 M	.....	.....	106.8	16.34	...
Controls, paraffin only	20 M	.....	.....	No tumors		

* Coward-Burn rat unit.
† Cole-Saunders rat unit.
‡ Daily amount necessary to maintain completely adrenalectomized rat, as defined by D'Amour and Funk (2).
§ A barely maintaining dose of cortin was given, the amount and schedule depending upon the condition of the individual animal.
‖ Injection schedule varied, depending upon the condition of the animal.
¶ Injection schedule varied, depending upon blood sugar.

Table II: 1,2,5,6-Dibenzanthracene

Group *	Number and sex of rats	Hormone dose per week	Injection schedule	Mean latent period, in days	σ	Critical ratio $\left(\frac{D_m}{\sigma_D}\right)$
Hyper-estrin	5 M	30 r.u.	3 × weekly	266	58.13	1.29
Hyper-estrin	5 F	30 r.u.	3 × weekly	287	25.43	4.30
Controls	5 M	.....	.....	238	45.64	...
Controls	5 F	.....	.....	246	36.51	...

* In the hyper-estrin groups all rats developed tumors; in the controls, only 4 out of each 5.

means of endocrine derangement. It was felt that if certain endocrine influences resulted in a deviation from the usual rate of tumor growth, new avenues of research would be opened up. This is, therefore, only an exploratory study in which a large number of experimental procedures were employed, rather than a thorough study of any one treatment.

It is generally conceded that certain types of chronic irritation are factors in the etiology of many cancers; *i. e.,* epithelioma of the lip and tongue, chimney sweep tumors, etc., so that a tumor produced by prolonged chemical irritation is somewhat analogous to some types of human neoplasms. Any influence capable of altering the pattern of development in such an experimental tumor might possibly also be responsible for similar action in spontaneous tumors of comparable origin. The rate of development was chosen as a criterion of influences acting upon the tumor because it can be measured with relative accuracy. The carcinogen used, methylcholanthrene, produces 100 per cent tumor formation in the Denver strain of rats, with considerable uniformity of growth. The mean latent period of tumor production in normal animals was 106.8 days. Consequently, any deviation from the usual development, as compared with an adequate number of control animals, could be traced to a known hormone which had been administered or withheld under controlled conditions.

The choice of the endocrine glands as a possible influence on rapidly growing tissue is based on a number of points, both proved and theoretical. Although their functions, in broad outline, are known, the mechanism of hormonal action remains obscure. Thus, while the growth-stimulating effect of the gonadal hormones upon the accessory sex structures is recognized, their mode of action is unknown. If the responsiveness of cells forming the accessory structures is an inherent property of these cells, it is possible that other (tumor) cells, peculiarly susceptible to some unknown growth-stimulating factor might also be responsive. The high incidence of cancer of the prostate, uterus, and mammary glands must not be overlooked in this connection. A similar argument can be advanced for the chorionic gonadotropins, present in some species during the time of rapid growth of the fetus, and for the more generally acting growth-promoting factor of the pituitary. The influence of the thyroid, pancreas, and adrenal medulla on general and carbohydrate metabolism indicates their possible importance. Finally, the anterior pituitary and adrenal cortex present two structures whose functions are so complex, as measured by the number of hormones secreted and their known and suspected inter-relations with other glands, that speculation as to the possible effects of deprivation or excess of their secretions has wide limits.

Examination of the data reveals, however, that few of these plausible expectations were realized. We felt, in view of the drastic difference between opposing groups (complete deprivation compared with great excess) that deviations from the normal, to be significant, should be of considerable magnitude. The data were submitted to statistical analysis. On the side of decreasing the latent period; *i.e.,* of stimulating the rate of tumor development, the results with the four groups treated with gonadotropins are statistically significant, $\frac{D_m}{\sigma_D}$ (critical ratio) being, in sequence, 2.7, 7.1, 2.5, and 2.5. Influence exerted in the opposite direction, toward retarding tumor growth, is significant for the hyper-cortin groups, $\frac{D_m}{\sigma_D} = 2.1$. It should be noted that these figures are significant in the statistical sense; that is, the differences observed are not due to errors in random sampling. It must be noted also, however, that it is admittedly difficult to say with certainty on just what day a tumor reached the standard mass of 1 cc., and therefore the experimental data on which the statistical analysis is based may be somewhat inaccurate. We do believe, in the case of the gonadotropins which are entirely consistent in all four groups, that these findings are suggestive enough to warrant further study with other dosages and other preparations of the same type. As for the remaining groups, the lack of influence on the development of tumors displayed by complete absence, or presence in excessive amount, of various hormones is interesting. When one contrasts the two thyroid groups, for instance, (thyroid secretion being a hormone having a long carryover effect from one injection to the next) and notes the close agreement between the latent periods, the conclusion is inescapable that this hormone is of no great significance in influencing the development of a growing tumor from a focus of chemical irritation. The same argument applies, with a little more variation in the results, to other hormones.

Records were kept of the time required from the beginning of growth; that is, the development of a tumor of 1 cc. volume, through progressive stages, until death of the animal. This period of maturation was subject to almost infinite variation within each group and seemed to depend upon which particular locus gained the ascendancy. This seemed to be simply a matter of chance. Usually only one, sometimes two, rarely more, of the injected sites developed into full-blown tumors, and again there was no consistency in any groups as regards this result. Usually, if the tumors tended to grow inward, especially from a locus on the abdominal wall, growth was rapid and death resulted early, although many animals lived

for months with tumors which, upon dissection, nearly equalled the mass of the animal itself.

All animals eventually died and were autopsied, records being taken of metastases. The percentage of grossly observable metastases in all groups was low, 10.5 per cent, with no significant variations between groups. The most frequent site of secondary invasion was the lungs.

A great deal of histologic material has been accumulated and is being studied. Sections of the endocrine system of tumor-bearing control animals were prepared to determine whether the tumor process itself had any influence upon the endocrine system. This is the reverse of the relationship to which the main study was devoted. Histologic sections were also prepared of the endocrine glands from representatives of each of the hormone-treated, tumor-bearing groups, in the hope that, should the tumor process produce a change in a given gland, the effect might appear to be more pronounced, or less pronounced, in the group to which that hormone had been supplied or from which it had been withheld. Studies of many tumors and metastases from different groups are in progress. This material will form the basis of another report.

Summary and Conclusions

An attempt was made to determine whether deficiency or excess of hormones would alter the rate of development of chemically induced tumors. The carcinogen used was methylcholanthrene in a 1 per cent paraffin solution, the total dose being 10 mgm., injected into 5 sites. Deficiency of each hormone was produced by extirpation of the gland involved; excess, by injection of the particular hormone. The rate of development was measured in terms of the elapsed time between injection of the carcinogen and the appearance of a tumor having a mass of 1 cc.

Neither deficiency nor excess of any hormone tested produced great deviation from the normal, although statistically significant differences, in the direction of increased rate of development, were shown by all groups of rats treated with gonadotropins. In the direction of decreased rate of development, differences were shown by the hypercortin group. Because of weaknesses in experimental technic, no stress is placed upon these statistical findings.

Considering the extreme physiologic contrast between animals completely deprived of a given hormone, as compared with animals receiving that hormone in excess, deviations from the normal were not large, under the conditions of the experiment. Since the dose of carcinogen used was capable of producing tumors in 100 per cent of the control animals, it may have been too large to be affected by the endocrine changes, whereas a more nearly border-line dose might have given the endocrine changes a better opportunity of exerting an influence.

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The Fibromatogenic Action of Specific Urinary Estrogens (Metahormones) in the Guinea Pig*†

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Conversion of estradiol and estrone, or ortho- and parahormones,[1] into estriol, a specific urinary metahormone of women, acquires a new interest since, in guinea pigs, toxic phenomena, including tumorigenesis, are elicited by prolonged action of follicular hormones. Further interest is added by the findings of Pincus and Graubard (20) who have reported that cancerous women appear to be unable to convert estrone into estriol to any appreciable extent. It is generally assumed that this conversion signifies inactivation; *i.e.,* that substances of high estrogenic activity (estradiol and estrone) are transformed into substances of low estrogenic activity (estriol in the urine of the pregnant woman; different urinary estrogens of the mare). One may also assume, tentatively, that by means of this conversion the body protects itself against the toxic or tumorigenic action of follicular hormones. This assumption was tested by making a quantitative comparison of the faculty of different ovarian and urinary estrogens to elicit abdominal serosal fibroids in the guinea pig.

Materials and Methods

Alpha-estradiol and estrone were used as representatives of ovarian estrogens. Both are found also in the urine, especially estrone, which can be extracted from the human placenta, as shown by Doisy (2) and Marrian (19). Since both are present in the ovary (2), they can be considered as primary ovarian estrogens. Estriol and equilenin were used as representatives of urinary estrogens. The first is present also in the placenta, but both are absent from the ovary. They can be considered as specific urinary estrogens.

Pellets of crystalline hormones were prepared by compression in a suitable hand press as described in detail in the thesis of Thibaut (23). The weighed pellets were implanted subcutaneously in castrated guinea pigs. After the death of the animals the pellets were recovered and weighed again. The animals were killed and autopsied at different intervals after implantation of the pellet. The fibrous reaction was classified in accordance with criteria specified in previous papers by Lipschütz and Vargas (14), and Lipschütz, Bellolio, Chaume, and Vargas (10). The results with respect to absorption and total tumoral effect as obtained in 114 animals are summarized in Table I and Fig. 1.

Comparative Fibromatogenic Activity of Different Estrogens

All of the four estrogens were tumorigenic. The localization of tumors was the same as in animals receiving estradiol and its esters, or artificial estrogens, as reported previously by Lipschütz and Iglesias (10), Iglesias (6), Lipschütz, Iglesias, and Vargas (11), and Szabo (22). However, there were considerable differences as to the degree of the reaction.

The first manifestations were found as early as 21 to 30 days after implantation of pellets. These were small, barely visible, nodules on the spleen, the stomach, and sometimes at the junction between the uterus and the parametrium. These primary manifestations of a fibrous reaction graded as 0.5 were present with all four estrogens.

The tumoral reaction increased with time. At 80 and 120 days marked differences became evident between estradiol and estrone on one hand, and estriol and equilenin on the other. As noted in our previous reports (6, 11, 15), individual variations were con-

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Crystalline estradiol was supplied by Dr. Carl Miescher, of the firm of Ciba in Basel. Estrone, estriol, and equilenin were supplied by Dr. Oliver Kamm, of Parke, Davis & Co.

† The senior author is responsible for the interpretation of results and preparation of the manuscript.

[1] In recent years Lipschütz (8) has used a new Spanish terminology to indicate the physiological relationships of the different sex hormones. According to this terminology estradiol, progesterone, and testosterone are *orthohormones;* estriol and the specific urinary estrogens of the mare, pregnandiol, androsterone, and dehydroandrosterone are *metahormones.* The term *parahormones* may be appropriate for those hormones whose physiological activity is doubtful; for example, estrone. The term *prehormone* also may be useful. It must be left to others to judge whether this terminology is convenient in English.—Authors' note.

siderable. Resistant animals were present in the groups given each of the four estrogens. Nevertheless, it was a striking finding that, in experiments continued for 50 days or longer, 6 out of 33 animals treated with estradiol and estrone developed high tumoral reactions, it is known that estrone is absorbed more slowly than estradiol. In our experiments, results of which are shown graphically in Figs. 1 and 2, we found that the absorption of estrone was considerably slower than that of estriol or equilenin. Nevertheless, the tumori-

TABLE I: TUMORAL EFFECT IN 71 CASTRATED FEMALE GUINEA PIGS (210 TO 530 GM. WHEN RECEIVING THE IMPLANT) WITH SUBCUTANEOUSLY IMPLANTED PELLETS OF FOUR DIFFERENT ESTROGENS

Estrogen	Duration of experiment in days	Number of animals	Average absorption per day μgm.	Total tumoral effect: Average	Total tumoral effect: Range	Number of animals with total tumoral effect not less than grade 6	
Estradiol	50	10	75	2.7	0-9	1	
Estradiol	80	5	18	4.6	0-12	1	6 out of 33
Estrone	50	5	13	3.2	0.5-8	1	
Estrone	80	5	11	3.9	0.5-6.5	1	
Estrone	120	8	5.5	4.6	1.5-12	2	
Estriol	50	4	44	1.9	0-5	0	
Estriol	80	5	20	2.4	0.5-4	0	
Estriol	120	8	15	3.1	0-8	1	1 out of 38
Equilenin	50	5	22	0.5	0-1.5	0	
Equilenin	80	9	19	0.7	0-4	0	
Equilenin	120	7	15	0	....	0	

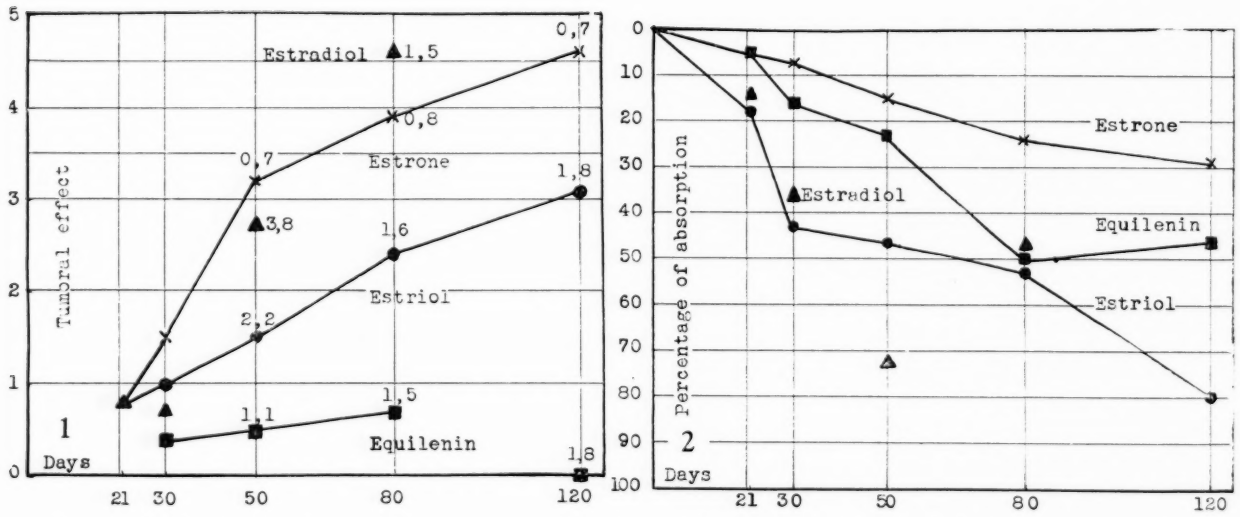

FIG. 1.—Graphs showing degree of fibromatogenic action of estradiol, estrone, estriol, and equilenin in 114 castrated guinea pigs. The estrogens in pellets were implanted subcutaneously. Each point is the average of results in 4 to 10 animals. The numbers near each point indicate the average absorption in mgm. The fibromatogenic action of estriol and equilenin is considerably less than that produced by equal or smaller quantities of estrone or estradiol. For details see Table I and Thibaut (23).

FIG. 2.—Graphs showing percentage absorption from subcutaneously implanted pellets of estradiol, estrone, estriol, and equilenin in 114 female guinea pigs. All pellets were of the same diameter, 1.6 mm., but not of the same length. The weights of the pellets varied between 1.1 and 6.2 mgm. Rate of absorption of estrone was less than that of other estrogens. For details see Thibaut (23).

not lower than grade 6, while among 38 animals treated with estriol and equilenin only one developed such a reaction, the tumoral reaction generally failing to appear.

The differences in the degree of tumoral reaction were not due to the differences in the quantities of hormones absorbed. From the work of Deanesly (1) genic action was enormously superior with estrone. Our results show, therefore, that specific urinary estrogens are less fibromatogenic than ovarian estrogens. This confirms the statement of Lacassagne (7) who found equilenin to be less active than estrone in eliciting mammary adenocarcinoma in mice, although no exact quantitative data on this point are available.

Comparative Hysterotropic Action

The question arises as to whether the lower fibromatogenic action of certain estrogens is concomitant with a lesser estrogenic activity. According to general opinion this should be so because, in the Allen-Doisy test, estriol and the urinary estrogens of the mare are less active than estradiol or estrone. The same results are obtained with the uterine tests. When rats are injected once daily for 5 days with estrogens in oil, the highest uterine ratio (ratio of the weight of the uterus in mgm. to body weight in gm.) is obtained with estradiol. With estrone this ratio is somewhat less and with estriol and equilenin it is considerably below the value with estradiol, as shown by the work of Dorfman (3). Similar statements concerning this ratio in the mouse were made by Evans, Varney, and Koch (5). Emmens (4), however, discovered that the differences in the degree of estrogenic effect exerted by the natural estrogens, as evidenced by cornification of the vaginal mucosa, depend in part upon the number of injections made into the same animal for purpose of assay. With two injections the approximate amounts of estradiol, estrone, and estriol in oily solution needed to produce 50 per cent of positive estrous vaginal responses are 1:4:280, whereas with four injections these amounts are only 1:2.7:6.4.

These findings of Emmens raise the question as to whether there might be equality in certain actions of estrogens, hitherto different as judged by the results of the Allen-Doisy test and the results of the short-term uterine test, provided a steady flow of hormone be maintained from a given source. Our results with pellets of estrogens appear to be in favor of such an assumption. In our experiments with pellets there was no considerable difference in uterine weights between estradiol, estrone, and estriol. Uterine weights were inferior with equilenin, but the difference between equilenin and the other estrogens on this basis was not so remarkable as the differences between the fibromatogenic actions, data on which are shown in Figs. 1 and 3.

It must be emphasized that, in experiments in which treatment with estrogens is prolonged, the uterine weight does not depend entirely upon an increase in the muscular coats. The increase depends to a large extent also upon the atypical proliferation which the endometrium undergoes under these experimental conditions. In some instances adenomatous polyps may fill the uterine cavity and descend into the cervix and vagina (18). Although we have not yet made a comparative microscopical study of the myometrial and endometrial reactions following implantation of pellets of different estrogens, we have noted that atypical growth of the endometrium, with the formation of polyps, may be obtained also with equilenin. Genital bleeding occurred with equilenin as with other estrogens.

Comparative Fibromatogenic Action of Follicular Hormones Absorbed from Pellets and Injections

In previous experiments we (13, 21) found that 38 to 50 injections of 150 to 200 μgm. of estradiol, or 150 to 300 μgm. of estrone, in oily solution; *i.e.*, a total of 5.7 to 11.4 mgm. given in the course of 3 to 4 months, did not elicit abdominal fibroids in female guinea pigs. Only the first manifestations of a fibrous reaction occurred in the form of tumoral seeds or fibrous strands at various places. Fibroids were produced only when 400 μgm. were injected thrice weekly; *i.e.*, when a total of 16 to 20 mgm. were injected in the course of 3 to 4 months. The results of the experiments we are now reporting provided a basis for comparison of the fibromatogenic action of estrogens administered on the one hand in repeated injections of oily solutions, and on the other by subcutaneous implantation of pellets.

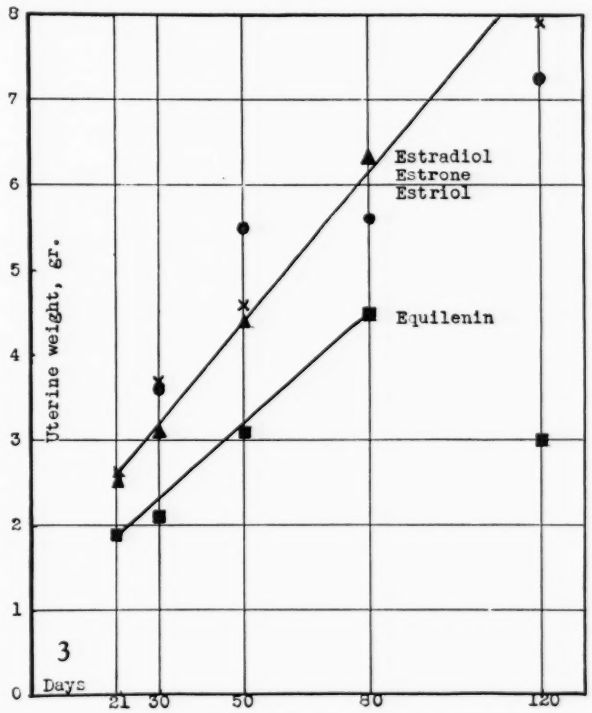

Fig. 3.—Graphic summary of uterine weights in 93 castrated female guinea pigs treated with estradiol, estrone, estriol, and equilenin. There was no difference between estradiol, estrone, and estriol. The averages were smaller with equilenin, but the differences in uterine weights were not as striking as the differences in fibromatogenic effects. Compare with Fig. 1.

In Table II we have summarized the results obtained in experiments with 18 guinea pigs killed 80 to 120 days after implantation of the pellet. These experiments are fully comparable as to duration of treatment with experiments on a group of 17 animals previously described in Tables I and II of the appendix of the report by Rodríguez (18), each animal in this group having received 40 to 50 injections of 150 to 300 μgm. of hormone in 95 to 123 days. As is shown in Table II, and Figs. 4A, 4B, and 4C, considerable tumorigenesis was elicited with pellets when only 15 μgm. of estradiol were absorbed daily in the course of 80 days.

assumed by Lipschütz (9) that the estrogen level in the blood will drop rapidly below the tumorigenic threshold unless enormous quantities are injected repeatedly. On the contrary, when there is a continuous supply of estrogen from a subcutaneously implanted pellet it is very likely that the level of concentration of estrogen in the blood is maintained constant. The height of this level will probably depend on the quantity of estrogen absorbed in unit time; *i.e.*, on the surface area of the tablet or pellet, as indicated by the studies of Lipschütz and Vargas (15, 16). The quantity sufficient to maintain the fibromatogenic threshold level of estrogens administered as pellets is

TABLE II: FIBROMATOGENIC ACTION OF SMALL QUANTITIES OF ESTRADIOL OR ESTRONE ABSORBED FROM SUBCUTANEOUSLY IMPLANTED TABLETS IN 18 CASTRATED FEMALE GUINEA PIGS

Series XXVIII guinea pig No.	Duration of experiment in days	Weight of pellet mgm.	Quantity absorbed		Total tumoral effect	Tumoral class			
			Total mgm.	Per day μgm.		Uterine subserous and parametrial	Uterine apical	Digestive tract and parietal	Splenic
Estradiol									
96 (Fig. 4, A-C)	80	2.2	1.2	15	12.0	3 pm.	3	3 pr., ms.	3
97	80	3.3	1.2	15	5.0	0	1	3 pr.	1
98	80	2.4	1.4	18	0.0	0	0	0	0
102	80	3.4	2.2	28	3.0	2 ss.	0	0	1
95	80	4.4	2.4	30	3.0	0	3	0	0
Estrone									
67	80	1.9	0.3	4	4.0	0	3	0.5 f., ms.	0.5 f.
69	80	2.1	0.3	4	0.5	0	0.5 f.	0	0
60 (Fig. 5)	80	5.2	0.4	5	6.5	0	3	3 ms.	0.5 f.
24	80	3.3	1.1	14	4.5	1 pm.	3	0.5 f., ms.	0
22	80	5.2	2.1	27	4.0	0	3	0.5 f., ms.	0.5 s.
74	120	2.1	0.2	1.7	2.0	0	0.5 f.	0.5 f., ms.	1
86	120	1.7	0.4	3.4	1.5	1 pm.	0.5 f.	0	0
70 (Fig. 6)	120	1.8	0.5	4.2	3.0	0	3	0	0
71	120	3.0	0.5	4.2	2.5	0	0.5 f.	0	2
85	120	1.7	0.6	5.6	3.0	1 ss., pm.	1	0	1
68 (Fig. 7, A and B)	120	1.9	0.9	7.5	4.0	2 ss., pm.	1	1 int., ss.	0
72 (Fig. 8)	120	2.4	0.9	7.5	12.0	3	3	3	3
66 (Fig. 9, A-C)	120	3.8	1.3	10.9	9.0	3 ss., pm.	3	1 pr.	2

Abbreviations: f. = fibrous strands; s. = tumoral seed; ss. = subserous tumors of uterus or intestine; pm. = parametrial tumors of uterus; ms. = mesenteric tumors; int. = tumor on intestine.

With pellets of estrone, 5 to 7.5 μgm. per day were sufficient to produce large fibrous tumors in the course of 80 to 120 days. The distribution and sizes of these tumors are indicated in Figs. 5 to 9C.

The comparative results obtained with implanted pellets and with injections of oily solutions demonstrate the fundamental importance of timing in tumorigenesis elicited by estrogens. Quantities as small as 15 μgm. of estradiol or 8 μgm. of estrone, or less, absorbed daily over a period of time, were equivalent in their fibromatogenic action to 400 μgm. absorbed from an oily solution injected every second day. The following explanation is suggested: Estradiol and estrone when given by subcutaneous injections are rapidly absorbed and partly inactivated in the liver. Hence injections every second day are not likely to maintain a stable level of estrogen in the body. Accordingly, it has been evidently much smaller than when these hormones are administered by injection.

These results provide new evidence that the estrogen exerts fibromatogenic action when experimental conditions provide for its *continuous* action on the effector tissues. Subcutaneous implantation of tablets or pellets is the most effective method for obtaining this effect. Lipschütz and his associates (17) have shown also that esterification, especially with caprylic acid in the 17- position, is another important means of securing continuity of action.

In contrast with the effects of 5 to 8 μgm. of estrone, absorbed from pellets, quantities of equilenin twice or three times greater, administered in the same manner, had no tumorigenic action or only an insignificant effect (Table I).

Fig. 4A.—Photograph showing multiple parametric tumors of both uterine horns, subserous uterine tumors of the left horn chiefly along the ventral muscular ridge, apical uterine tumors, large tumor at the hilum of the spleen, tumors on the surface of the spleen, tumors of the epiploon. These developed in a castrated female guinea pig (Table II, No. 96) with a subcutaneously implanted pellet of 3.2 mgm. of estradiol. Absorption was at the rate of 15 μgm. daily, or 2.2 mgm. of estradiol in 80 days.

Fig. 4B.—Photograph of the tumoral masses between the stomach and the spleen in the same animal as in Fig. 4A.

Fig. 4C.—Photograph of three tumors of the mesocolon and, at the left, a large tumor of the parietal serosa, in the same animal as in Fig. 4A.

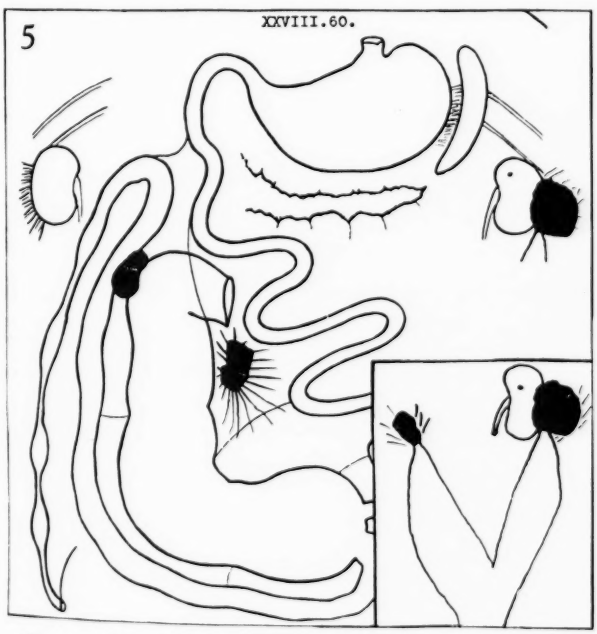

Fig. 5.—Diagram showing tumors at the apices of the uterine horns and at other sites in the abdominal cavity in a castrated guinea pig (Table II, No. 60) treated by implantation of a pellet of 5.2 mgm. of estrone. Only 0.4 mgm. was absorbed in 80 days, or 5 μgm. daily.

Summary and Conclusions

The ovarian estrogens, estrone and estradiol, and the specific urinary estrogens, estriol and equilenin, were implanted subcutaneously, in the form of pellets, into castrated female guinea pigs. Observations were made upon the rates of absorption and the comparative effects of these estrogens upon uterine weights and their fibromatogenic activities.

Specific urinary estrogens, such as estriol and equilenin, elicited abdominal serosal fibroids similar to those following subcutaneous implantation of pellets of estradiol and estrone. The fibromatogenic action of estriol was less than that of estradiol and estrone. The fibromatogenic action of equilenin was insignificant.

The stronger action of estradiol and estrone as compared with that of the specific urinary estrogens was not due to absorption of larger quantities of the ovarian estrogens. On the contrary, estrone was absorbed more slowly and was more effective than twice the quantities of absorbed estriol and equilenin.

Estriol produced increase of uterine weight almost equal to that produced by estradiol and estrone; the

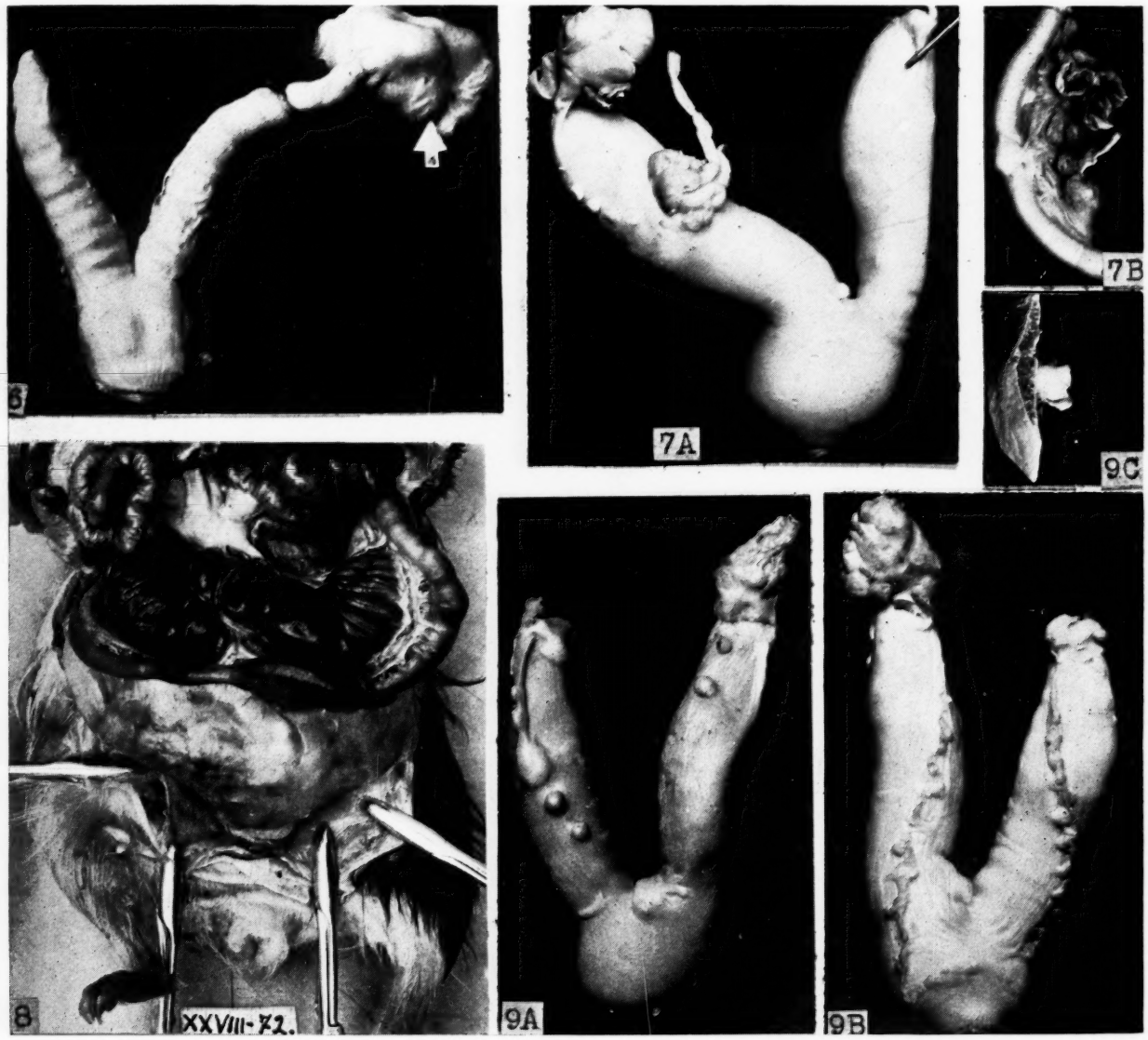

FIG. 6.—Photograph showing a large tumor at the apex of the left uterine horn in contact with the abdominal wall in a castrated guinea pig (Table II, No. 70) implanted subcutaneously with a pellet of 1.8 mgm. of estrone. Absorption was at the rate of 4.2 μgm. daily, or a total of 0.5 mgm. in 120 days.

FIG. 7A.—Photograph of several subserous uterine tumors, an apical tumor of the right uterine horn, and adhesions between the tumors and the omentum, in a castrated female guinea pig (Table II, No. 68) implanted with a pellet of 1.9 mgm. of estrone. Absorption was at the rate of 7.5 μgm. daily, or a total of 0.9 mgm. in 120 days.

FIG. 7B.—Photograph of a tumor of the mesocolon in contact with the serosa of the intestine, in the same animal as in Fig. 7A.

FIG. 8.—Photograph of fibrous tumors in castrated female guinea pig (Table II, No. 72) implanted subcutaneously with a pellet of 2.4 mgm. of estrone, showing tumors in the mesentery of the ileum and uterus embedded in large tumoral masses to which the colon is firmly adherent. Absorption was at the rate of 7.5 μgm. daily, or a total of 0.9 mgm. in 120 days.

FIG. 9A.—Photograph of the ventral surface of the uterus of a castrated female guinea pig (Table II, No. 66) implanted subcutaneously with a pellet of 3.8 mgm. of estrone, showing several subserous uterine tumors at typical locations. Absorption was at the rate of 11 μgm. daily, or 1.3 mgm. in 120 days.

FIG. 9B.—Dorsal view of the uterus shown in Fig. 9A, showing a chain of parametrial fibroids.

FIG. 9C.—Tumor at the hilum of the spleen in the same animal as in Fig. 9A.

increase with equilenin was smaller. The differences between the hysterotropic actions were less significant than the differences between the fibromatogenic effects.

When a steady flow of estrogen is established from a subcutaneously implanted pellet, abdominal fibroids can be elicited with about 5 μgm. of estrone absorbed per day, as compared with 400 μgm. injected thrice weekly in the course of 4 months. This difference is explained by the assumption that the tumorigenic faculty of estrogens is dependent upon a continuous action upon the effector tissues.

Under the same experimental conditions which provided for continuous action quantities of equilenin, two or three times as large as the effective amounts of estrone, had no fibromatogenic action or only an insignificant effect.

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Carcinogenic Effect of Estradiol and of Theelin in Marsh-Buffalo Mice*

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The present report is concerned with a comparison in Marsh-Buffalo mice of the action of estradiol with that of theelin when injected over a long period and in massive doses. Studies performed in this laboratory indicate that the Marsh-Buffalo strain is highly susceptible to mammary cancer and markedly resistant to the carcinogenic effect of exogenous theelin. Since the relation between estradiol and theelin in catabolism is not known, it appeared desirable to ascertain whether estradiol would be more effective, and whether the intact ovary inhibited the influence or acted synergistically to exogenous estradiol.

Suntzeff *et al.* (5) state, "In strain New Buffalo nonbreeding mice injected with estrin the percentage of tumors developing was relatively low, although it was somewhat higher than in nonbreeding control mice. There is thus some indication that the mammary gland of New Buffalo mice is relatively resistant to the stimulating action of estrin." Since these workers used only 2 to 7 mice per experiment, this work must be regarded as preliminary in nature. We were, therefore, prompted to repeat these experiments on a larger scale, since resistance to carcinogenesis caused by theelin in a high cancer strain would be a phenomenon which could not readily be ignored. The results of our experiment published to date (1), in which crystalline theelin was used, confirmed the original suggestion of Suntzeff *et al.* that the mammary gland of the Marsh-Buffalo mouse is relatively resistant to the stimulating action of estrin. The incidence of mammary cancer in our control Marsh-Buffalo mice parallels the data originally published by Marsh (3) and confirmed by Murray and Hoffman (4). It should be noted that the data of Suntzeff *et al.* indicate a considerably lower cancer incidence, and in this respect only is there a divergence in results. Since the studies reported in this paper were completed, Suntzeff *et al.* (6) have published additional data on the Marsh-Buffalo strain, and conclude that the estrogen dosage raised the incidence of tumors in nonbreeding mice to approximately that of the breeders. This deduction is obviously unwarranted as the standard deviation of the mean for the number of animals used equals the difference. In our opinion, the data again demonstrate that mice of the Marsh-Buffalo strain are highly resistant to estrogen carcinogenesis, and justify the characterization of this strain as having a relatively low incidence of cancer in Dr. Loeb's laboratory.

Experimental

At 22 days of age, litter-mate females were divided into 3 groups of 40 mice per group. One group served as a control and received injections of sesame oil. Another group received injections of estradiol.[1] The third group was ovariectomized at weaning, and received estradiol in the same amount as did the group of intact mice. The amount of estradiol administered in these experiments corresponds to the larger amounts of theelin administered in our previous experiments with theelin (1). The estradiol was dissolved in sesame oil (2 mgm. per cc.) and was administered intramuscularly or subcutaneously in doses of 0.04 cc. twice weekly for a period of 6 months. Each surviving mouse received a total dose of 3.3 to 4.2 mgm. estradiol. The controls received 0.04 cc. sesame oil twice weekly over a 6-month period. The first injections were given at the age of 1 to 2 months.

Since the purpose of these experiments was to compare the action of estradiol with that of theelin, the data of experiments with mice receiving theelin, which have been published (1), are retabulated and compared with experiments in which estradiol was used. Forty mice received theelin in each experimental group. In Tables I and II are tabulated the cumulated percentage incidences of adenocarcinoma of the breast, lymphosarcoma, spontaneous death, and combined influences removing animals from the experiment. Spontaneous death is used as a measure of toxicity. At autopsy, some of these animals were found to have lymphosarcoma. With the development of a palpable tumor, the animal was removed from the experimental group and sacrificed after the tumor had developed in diameter to 1.5 cm.

Results

Toxicity.—In the controls of both the theelin and estradiol series, the 13 and 12 per cent incidence (cumulative) of death before the 7th month is higher than is usually encountered for control mice. In a series of 120 virgin female mice housed and fed under strictly analogous conditions, but not given sesame oil, only 1 mouse died in a similar period. A slightly toxic effect of the sesame oil is indicated. In the same 7-month period, 35 per cent of the theelin-dosed mice,

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[1] The authors are indebted to the Schering Corporation for the supply of alpha estradiol.

22 per cent of the estradiol-dosed mice (intact), and 20 per cent of the estradiol-dosed ovariectomized mice died. The difference for the theelin-dosed mice is statistically significant (2.4 times the standard deviation of the mean). At a later date (the 10th month) the toxic effect of the estradiol, particularly in the series of intact mice, is also clearly indicated.

Lymphosarcoma.—The incidence of lymphosarcoma is definitely increased by estradiol dosage in both intact and ovariectomized mice. The increase in the theelin-dosed mice is doubtfully significant. The maximum increase in the incidence of lymphosarcoma over the controls for the theelin-dosed mice is 15 per cent, 28 per cent for the estradiol-dosed intact mice, and 47 per cent for the estradiol-dosed ovariectomized mice. However, analysis of the data does not warrant the conclusion that estradiol enhances lymphosarcoma formation over that produced by theelin. In the case of the ovariectomized mice, the low incidence of breast tumor formation gave an opportunity for more mice to develop lymphosarcoma.

Adenocarcinoma.—According to the data of Marsh, 66 per cent of 70 virgin Marsh-Buffalo mice developed tumors by the 16th month. Our own data based on 6 groups of mice, each group comprising 30 to 50 mice, are as follows: 67, 62, 63, 63, 70, and 52 per cent respectively. Within the expected normal variation our data confirm the original data of Marsh. In our present experiments 45 per cent of the controls for the theelin-dosed group and 28 per cent of the controls for the estradiol-dosed mice developed mammary tumors in a comparable period. This decrease and variation in incidence may be attributed to the injected sesame oil. The importance of controlling this factor is indicated.

A comparison of the data in Tables I and II shows that there is a doubtfully significant increase in tumor incidence in the theelin-treated mice as compared with controls and no significant difference for either the intact or ovariectomized estradiol-treated mice as compared with controls.

Since the cumulative per cent incidence of adenocarcinoma, as shown in Tables I and II, may not be a true measure of the influence of the estrogens in enhancing this form of carcinoma because of the compli-

TABLE I: CUMULATIVE INCIDENCE OF ADENOCARCINOMA OF THE BREAST, LYMPHOSARCOMA, AND SPONTANEOUS DEATH IN 40 CONTROL AND 40 THEELIN-DOSED MARSH-BUFFALO VIRGIN FEMALE MICE

	Theelin-dosed				Control			
Age in months	Per cent adeno-carcinoma	Per cent lympho-sarcoma	Per cent sponta-neous death	Per cent removed from experiment	Per cent adeno-carcinoma	Per cent lympho-sarcoma	Per cent sponta-neous death	Per cent removed from experiment
3-6	..	8	27	27	..	3	8	8
7	3	10	35	38	0	5	13	13
8	10	10	38	48	0	8	15	15
9	20	18	43	65	5	8	20	25
10	25	20	45	72	13	8	20	32
11	33	23	45	83	18	13	25	45
12	38	23	45	88	20	13	25	48
13	38	28	50	93	23	13	25	50
14	38	28	50	93	35	15	25	65
15	40	28	50	95	43	18	25	75
16	..	..	..	..	45	..	..	78

TABLE II: CUMULATIVE INCIDENCE OF ADENOCARCINOMA OF THE BREAST, LYMPHOSARCOMA, AND SPONTANEOUS DEATH IN 40 CONTROL AND 40 ESTRADIOL-DOSED MARSH-BUFFALO VIRGIN FEMALE MICE

	Intact estradiol-dosed				Ovariectomized estradiol-dosed				Control			
Age in months	Per cent adeno-carci-noma	Per cent lympho-sarcoma	Per cent spontane-ous death	Per cent removed from ex-periment	Per cent adeno-carci-noma	Per cent lympho-sarcoma	Per cent spontane-ous death	Per cent removed from ex-periment	Per cent adeno-carci-noma	Per cent lympho-sarcoma	Per cent spontane-ous death	Per cent removed from ex-periment
3-6	..	..	12	12	..	8	10	15	..	..	8	8
7	3	5	22	25	..	10	20	25	3	..	12	15
8	5	10	32	40	3	18	25	35	3	..	12	15
9	13	18	42	58	5	25	28	45	3	..	15	18
10	13	28	48	68	8	33	32	55	5	..	15	20
11	15	28	48	70	10	33	32	60	5	..	15	20
12	20	28	48	75	15	38	38	70	15	3	15	36
13	20	28	48	75	15	38	38	70	18	3	15	38
14	25	28	48	80	15	48	38	80	22	3	15	43
15	25	28	48	80	15	50	38	85	22	3	15	46
16	25	28	48	80	15	50	38	85	28	8	15	50
17	25	30	48	..	15	50	38	..	28	10	15	..

cating factors of death (toxic reaction) and development of lymphoid tumors, the incidence of carcinoma development on the basis of surviving animals should be considered. Using surviving animals as a basis of comparison, the calculation (Tables III and IV) indicates that at the 9th month there is a significant increase in tumor incidence in the theelin-dosed mice, 34 contrasted with 7 per cent, a doubtfully significant increase in the estradiol-dosed intact mice, 18 contrasted with 3 per cent, and no increase in the estradiol-dosed ovariectomized mice. At the 10th month, the increase for the theelin-dosed mice is probably significant, 48 per cent contrasted with 17 per cent, while the differences for the estradiol-dosed mice, 18 and 12 per cent contrasted with 6 per cent, are not significant. At a later date the data for estradiol-dosed intact mice indicate an increased incidence over the controls. In the case of the estradiol-dosed ovariectomized mice this difference never becomes significant.

Influence of estradiol on mammary glands.—Mounts of whole mammary glands were prepared from 5 mice of each experimental group, at the age of 10 to 12 months. The alveolar ducts in 5 control mounts were not as wide as the ducts in 5 similar mounts made from mice which had received estradiol. This difference was marked; the number of ducts was not increased. In the control mice the alveoli were for the most part rudimentary, but in 2 of the 5 dosed mice the alveoli were well developed in some areas. In 5 mounts of breasts of castrated mice, which had received estradiol, the alveolar ducts were slightly less numerous than those of the control group and the ducts were slightly wider. In 2 out of 5 mounts the alveoli of the castrated mice were more developed.

Discussion

We are unable to account for the discrepancy between the tumor rate of the Marsh-Buffalo strain as observed in the laboratory of Loeb and in the Buffalo and Santa Barbara laboratories. A similar discrepancy for their strain A mice was observed by Loeb and his associates at St. Louis. They do not think it probable that the nature of the diet is the responsible factor. The relatively high incidence of lymphosarcoma in our Marsh-Buffalo mice is not described by others who maintain this strain, though Marsh noted its occurrence. Since our data indicate that the injection of sesame oil alone, which was slightly toxic, inhibited the rate of tumor appearance, the importance of the general well-being of the mice is clearly indicated. In the Marsh-Buffalo strain, this factor may assume proportions as great as any effect produced by exogenous

Table III: Per Cent Breast Tumors Developing on Basis of Surviving Mice

Age in months	Theelin-dosed				Control			
	Number tumors	Number mice surviving	Per cent tumors	Cumulative per cent	Number tumors	Number mice surviving	Per cent tumors	Cumulative per cent
3-6	..	29	..	..	..	37	..	..
7	1	25	3	3	..	35	..	..
8	3	21	12	15	..	34	..	..
9	4	14	19	34	2	30	7	7
10	2	11	14	48	3	27	10	17
11	3	7	27	75	2	22	7	24
12	2	5	28	..	1	21	5	29
13	0	3	0	..	1	20	5	34
14	0	3	0	..	5	14	25	59
15	1	2	33	..	3	10	21	80
16	..	..	..	..	1	9	..	..

Table IV: Per Cent Breast Tumors Developing on Basis of Surviving Mice

Age in months	Intact estradiol-dosed				Ovariectomized estradiol-dosed				Control			
	Number tumors	Number mice surviving	Per cent tumors	Cumulative per cent	Number tumors	Number mice surviving	Per cent tumors	Cumulative per cent	Number tumors	Number mice surviving	Per cent tumors	Cumulative per cent
3-6	..	35	..	..	..	34	..	..	..	37	..	..
7	1	30	3	3	..	30	..	..	1	34	3	3
8	1	24	3	6	1	26	3	3	..	34	..	3
9	3	17	12	18	1	22	4	7	..	33	..	3
10	0	13	..	18	1	18	5	12	1	32	3	6
11	1	12	8	26	1	16	6	18	..	32	..	6
12	2	10	17	43	2	12	13	31	4	26	13	19
13	0	10	..	43	..	8	..	..	1	25	4	23
14	2	8	20	63	..	6	..	..	2	23	8	31
15	..	..	..	..	..	..	..	..	0	22	0	31
16	..	..	..	..	..	..	..	..	2	..	9	40

estrogens. It is obvious that control of this factor is of the greatest significance.

Earlier studies (1) showed that dosage of 60, 180, or 1,000 rat units of theelin per mouse during a 6- to 15-day period at the age of 50 to 90 days produced no discernible changes in the mammary glands of Marsh-Buffalo mice. Two thousand rat units of theelin brought about slight changes. It is apparent from the present studies that long-continued injections of massive doses of an estrogen produce the changes which others have succeeded in producing in other strains of mice in a relatively short period and with less material. It is also apparent that the intact ovary acts synergistically, and that in the absence of the ovary, estradiol alone is able to maintain both ductal and alveolar development.

Gardner (2) has shown that in male mice of the C3H strain mammary growth and tumor development are inhibited by large doses of estrogens, and increased by smaller doses. In this connection our theelin experiments previously recorded are of interest. The injection of 2.2 mgm. theelin per mouse in 8 months did not increase the incidence of cancer over controls or mice which received 1.1 mgm. theelin over the same period. The 2.2 mgm. dose was toxic, 43 per cent of the mice dying. In another experiment, the one retabulated in this paper, the 3.8 mgm. theelin per mouse was administered in the minimum amount of sesame oil. The toxicity was reduced and the rate of tumor appearance increased over that of controls which had received the same amount of oil.

The present status of research regarding carcinogenesis in the Marsh-Buffalo mice may be summarized as follows: 1. The mouse belongs to a high cancer strain as indicated by data (1, 3, 4) obtained in three laboratories, but to an intermediate cancer strain as indicated by data obtained in a fourth laboratory (5, 6). 2. The development of mammary tumors is influenced by the general well-being. 3. Increasing the incidence of breast tumor formation significantly by exogenous estrogens in nontoxic doses has not been accomplished (1, 5, 6). 4. Toxic doses of theelin or estradiol enhance development both of lymphosarcoma and of breast tumors (not clearly established in the case of breast tumors). 5. Formation of breast tumors may be produced in the ovariectomized mouse by estrogen dosage (toxic doses). 6. Success has not attended the production of mammary gland development by estrogen dosage which would compare with that produced by gonadotropin dosage in a short interval of time (1). 7. Gonadotropin dosage at intervals over a long period either has no influence (prolan) or significantly decreases the incidence of breast tumor formation (equine and sheep pituitary gonadotropin) (1).

While we have succeeded in enhancing breast tumor formation in the Marsh-Buffalo mouse this has not been accomplished without enhancing the development of lymphosarcoma. There is, therefore, nothing specific about the estrogenic carcinogenesis, certainly nothing to indicate that the breast is more responsive. It is not known whether tumor formation could be produced in ovariectomized mice by nontoxic doses of estrogens. In view of the negative response to such doses in both intact male and female mice in enhancing carcinogenesis, this information would be desirable.

Summary and Conclusions

Mice of the Marsh-Buffalo strain receiving 0.08 cc. weekly injections of sesame oil showed a lower tumor rate than did mice that were not so treated. A 12 to 13 per cent incidence of early death indicated a toxic effect.

In groups of 40 mice, 35 per cent of theelin-dosed mice and 20 per cent of estradiol-dosed mice died within a 7-month period. Approximately 4 mgm. of estrogen were administered per mouse in a 6-month period.

Estradiol was not more toxic in ovariectomized mice than in intact mice and not more toxic than theelin, on a weight basis.

The incidence of lymphosarcoma was definitely increased by estradiol dosage in both intact and ovariectomized mice. The increase in the theelin-dosed mice was doubtfully significant.

On the basis of cumulative percentage incidence, theelin produced a doubtfully significant increase and estradiol no significant increase in breast tumor formation as compared with controls. In ovariectomized mice estradiol maintained the rate at approximately that of the controls. When the incidence was calculated on the basis of surviving mice, a significant increase in tumor formation was produced in the intact treated mice but not in the series of ovariectomized mice treated with estradiol.

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Homoiotransplantation of Spontaneous Tumors into Mice Bearing Spontaneous Tumors*

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In 1907, Loeb (5) observed that when a mouse bearing a spontaneous tumor was inoculated with a piece of spontaneous tumor from a second mouse, the transplanted piece continued to grow until the time when the host died, 13 days after transplantation. Proliferating tumor tissue was seen on microscopic examination of the graft. On the other hand, when the same tumor was transplanted into a large number of normal mice, growth was not noted in any of these animals. Subsequently, however, Haaland (4) maintained that tumors which originated spontaneously in a mouse could be transplanted into normal mice just as well as into mice with spontaneous tumors. Later, Fleisher and Loeb (2) extended these investigations to include 55 mice with spontaneous tumors and 69 normal controls of similar strains, into all of which pieces of tumors were transplanted. Control mice and mice with spontaneous tumors were inoculated with pieces from the same spontaneous tumors obtained from mice of a different strain. In 16 of the experimental mice (29 per cent) the transplantation was successful and the grafted tumors grew, while in only 5 mice of the control group (7 per cent) did the tumor transplant proliferate.

These observations were further confirmed by subsequent experiments of Fleisher and Loeb (3), in which 82 mice with spontaneous tumors were inoculated with spontaneous neoplasms which had originated in other animals of this species. In 21 of these mice (26 per cent) the transplantation was successful, while in only 5 of 164 control mice (3 per cent) did the tumor grafts take.

While this might appear to be conclusive evidence that mice with spontaneous tumors offer a better soil for the growth of homoiotransplanted spontaneous tumors than do normal mice, it must be considered that variable factors may have entered into these experiments, and that the greater number of positive results in animals which were the bearers of spontaneous tumors may, after all, have been due to such variable factors. It was therefore thought advisable to repeat these experiments with different strains of mice and to compare the present findings with those previously reported by Loeb and Fleisher.

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Experiments

Fifty-two breeding female mice, bearing spontaneous tumors of the mammary gland, were inoculated with pieces of spontaneous mammary gland carcinoma from mice of different strains. Fifty-two normal breeding females from the same strain as the tumor-bearing, breeding mice were similarly inoculated. In most cases, the age of the control mice and of the experimental mice was about the same, and in no case did the difference in age between corresponding mice of each group exceed one month. Growth of a transplanted tumor was considered as successful when at least one diameter of the graft had increased to more than 1 cm., since transplants which attained this size did not retrogress. However, as we shall show below, some transplants of even smaller diameter contained proliferating tumor tissue, although these latter retrogressed. The results are tabulated in Table I, where it may be noted that successful growth of the tumor grafts did not develop in any of the control mice, while 11 of the 52 experimental mice (21.2 per cent) were successfully inoculated with spontaneous tumors of mice from other strains. This is in essential agreement with the experiments of Loeb and Fleisher.

In the last column of Table I we have indicated the ages of individual mice in which the transplantation of the tumor was successful; these mice all ranged between the ages of 8 and 11 months. Mice inoculated between the ages of 12 and 14 months did not develop tumors. If we consider, therefore, only mice under 12 months of age, the percentage of tumor takes would be 33.3 per cent, since 33 of the 52 experimental mice were in this age group (8 to 11 months), and in 11 of them active proliferation of tumor transplants occurred.

In the course of these investigations it was noted that in certain mice bearing both a spontaneous and a homoiotransplanted tumor, the transplanted tumor seemed to grow for a period of approximately 7 to 10 days, although it did not attain, during that time,

a sufficient size to be designated as a successful tumor take. The size of the transplant then remained stationary for about one week, after which it gradually retrogressed. On the other hand, homoiotransplantations of tumor tissue into normal mice resulted in a rapid retrogression of the graft, which was no longer palpable or visible within 4 to 6 days after transplantation. This initial growth and delayed retrogression was noted in 14 of the mice bearing spontaneous tumors, including 3 animals between the ages of 12 and 14 months. Microscopic examination of such a small tumor transplant after 10 days showed the presence of a considerable amount of living, well-preserved tumor tissue in the periphery of the transplant; an occasional mitosis was seen in the tumor cells. Surrounding the transplant there was a fibrous capsule, and a fibrous stroma also separated groups of tumor cells. The center of the transplant was mostly necrotic, although even here groups of apparently viable tumor cells were still present. After 15 days, the transplant was completely necrotic and was surrounded by a capsule of fibrous tissue.

The number of mice in which a temporary growth occurred was probably even greater than that noted above, since we did not take cognizance of the occurrence of such early proliferation until some time after these experiments had been in progress. In addition, there were a number of instances in which mice with slowly-growing tumor transplants died as a result of the rapid enlargement of the spontaneous tumor which they bore, at a time when the transplanted tumor had not yet attained a diameter of 1 cm. It is possible that some of these transplants either would have continued to grow, in which case they should have been included in the list of successful grafts, or they would slowly have undergone retrogression after a period of initial growth; some of these have been included in the fourteen cases designated as having exhibited temporary growth.

Discussion

These results are in remarkable agreement with those of Fleisher and Loeb (2, 3). As we have stated, in two separate groups of experiments they obtained 29 per cent and 26 per cent successful takes of homoiotransplanted spontaneous tumors in mice already bearing spontaneous new growths; our present data show a percentage of 21.1. On the other hand, Fleisher and Loeb observed that 7 per cent and 3 per cent, respectively, of normal mice showed successful homoiografts of spontaneous tumors, whereas in the present group none of the control mice was successfully inoculated.

If we combine all the available data, it can be noted that of 190 experimental mice bearing spontaneous neoplasms, 49 (26.5 per cent) were successfully inoculated with spontaneous tumors of other mice, while in at least 285 controls there were only 10 (3.5 per cent) successful implantations. In reality, the difference between the results of transplantation of tumors of the mammary gland in normal mice and in mice which are the bearers of spontaneous tumors is greater than these figures indicate. We have found in our series that in a number of instances the grafts show a temporary growth in the mice with spontaneous tumors, which is followed by retrogression, in contrast to their behavior in the normal mice, where there is no indication of growth as indicated by gross tests.

As Loeb has shown in a number of investigations (6-11), the fate of a transplant of either normal or tumor tissue primarily depends upon the specific adaptation between tissues and body fluids of host and transplant, and this is determined by the organismal and, in particular, by the individuality differentials of donor and host. The lack of adaptation between host and graft in transplantation between different strains is shown in the infrequent success in the transplantation of tumors from one strain to another strain of normal mice. In some way the presence of a spontaneous tumor in the host makes it possible for the transplanted tumor to overcome, in a certain percentage of animals, this lack of specific adaptation and to grow. We have reported previously (1) that in mice which are the bearers of large tumors anemia and loss of weight may develop, and these factors may distinguish such mice from the majority of normal mice. Nebenzahl (12)

Table I: Transplantation of Spontaneous Tumors of the Mammary Gland

Strain of the original tumor-bearing mouse and strain into which tumor was transplanted	Control mice			Mice with spontaneous tumors			Age of mice with successful grafts, in months
	Number of mice	Variation in age of mice, in months	Number of successful tumor grafts	Number of mice	Variation in age of mice, in months	Number of successful tumor grafts	
C3H to D	11	9-13	0	11	8-12	2	8, 9
C3H to A	12	8-12	0	12	8-12	4	8,* 10, 11
D to C3H	14	8-11	0	14	8-13	3	10, 11 *
New Buffalo to C3H	5	9-12	0	5	9-12	0	
CBA to D	10	9-13	0	10	8-14	2	10, 11
Totals	52		0	52		11 (21.2%)	

* Two mice in this age group. Other figures denote the age of an individual mouse with a successful tumor graft.

has made a similar observation in humans. However, it is not probable that these conditions play a role in favoring the growth of the homoiotransplanted tumor. In most of the present experiments the mice bearing spontaneous tumors were in good condition at the time of transplantation, although, as we have previously shown, anemia may in some cases already have become manifest in mice with small tumors.

As the age of controls and of mice with spontaneous tumors was about the same the difference in results cannot be referred to this factor. There is, however, a strong indication that the younger mice with spontaneous tumors provide more suitable conditions for the growth of a transplanted spontaneous tumor than do the older mice, and that the difference in age of the mice which are bearers of spontaneous tumors may then be the factor which determines whether a transplanted tumor grows or does not grow. It seems that in mice older than 11 months, the transplant does not grow permanently, notwithstanding the presence of a spontaneous tumor in the host.

In further experiments, it should be determined whether or not the presence of the spontaneous tumor of the host is an important condition for the growth of the graft, or whether the factors which led to the development of the spontaneous tumor are the essential conditions distinguishing bearers of tumors and control mice in their reaction to implanted tumors. In such experiments the host's tumor should be extirpated previous to the homoiotransplantation of another spontaneous tumor. No definite statement can therefore be made at present as to the reason why mice bearing spontaneous tumors are especially susceptible to transplants of spontaneous tumors developing in animals belonging to a different strain; but the possibility might be considered that in these hosts the presence of the spontaneous tumor either provides a favorable growth substance or that it neutralizes a substance inhibiting the growth of transplanted tumors.

Summary and Conclusions

1. Mice which are bearers of spontaneous tumors offer a better soil for the growth of transplanted spontaneous tumors which develop in mice belonging to different strains than do normal mice; they grow in about 22 per cent of the tumor-bearing mice, when they do not grow or grow only exceptionally in the controls.

2. There are additional mice bearing spontaneous tumors, in which the inoculated tumors grow temporarily and then regress, while no growth visible to the naked eye occurs in normal mice.

3. The successful growth of the transplants has so far been observed only in mice below the age of 12 months bearing spontaneous tumors, while in older mice no growth took place.

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The Influence of Syphilis in Cancer of the Cervix Uteri

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The problem of the influence of syphilis upon the neoplastic process in general has not been solved. Some investigators, on the one hand, have ascribed to syphilis an etiological role. Others deny any relationship between the two diseases. The consensus of opinion (2), however, is that an inter-relation exists between the two in the case of carcinoma of the buccal cavity, though the mode of interaction is not known. In addition to a causative role is the influence that syphilis may exert on the clinical course of a tumor through the personal equation involved or through some alteration in the pathology of the disease. Regardless of the lack of objective data bearing upon this subject there is a widespread impression among radiologists that when cancer appears in a syphilitic patient the prognosis is very much less favorable than in the absence of a spirochetal infection. In a recent article Rosh (5) has summarized the opinion of radiologists as follows: "We may state at this point, that patients who had a previous syphilitic infection show a tendency to earlier recurrence. It is our impression that this applies to the untreated syphilitic as well as to the treated ones, particularly in the younger age groups."

To a lesser degree the opinion is held among pathologists and clinicians that cancer is more highly malignant in the syphilitic patient. Black (1) concluded that "A final possibility is that in leaving syphilis untreated during the treatment of cancer the prognosis may be worsened, but I do not know of any evidence to this effect." Recently Schrader (6) reported 47 cases of cervical cancer occurring in women with positive serology for syphilis, a group representing approximately 4 per cent of his total series. In this group of 47 only 7 had had adequate antiluetic therapy. He concluded that syphilitic women, with or without adequate antisyphilitic therapy, respond poorly to radiation treatment and suggested that radiation morbidity from such causes as bladder and rectal distress and anemia is greater than in those free from syphilis.

The following investigation was undertaken to study this problem, using data obtained at the time a positive diagnosis of cervical uterine cancer was made. The symptoms, physical findings, and histopathology have all been tabulated and considered. No attempt has been made to include the results of treatment as this aspect will be dealt with in full at a later date. The data available do not give reliable information on the treatment given for syphilis in the 7 cases which had been diagnosed prior to the diagnosis of cancer. Three of them had received known efficient treatment for a period of 4 and 5 years. Unfortunately the question of leukoplakia and condylomas preceding the development of carcinoma in this series cannot be answered from our records.

CLINICAL MATERIAL

The data were derived from the records of 227 consecutive cases of epithelioma of the cervix uteri seen at the Los Angeles General Hospital and the Hornsby District Hospital in Sydney, Australia. The only factor of selection operating on the total series consisted of the restriction that the patients be unable to pay for treatment in private hands but this in no way affects the comparison within the group. A histopathologic confirmation of the diagnosis is available in each case, obtained either by biopsy or autopsy. Serologic examination of the blood was made in each patient and a positive complete fixation reaction was accepted as the criterion for the presence of syphilis. By this method 36 patients were considered to be syphilitic and 191 were classed as free from the disease. This gives a percentage incidence of 15.8. The total incidence of syphilis as indicated by routine blood tests in the hospital averages about 5 per cent but it cannot be used here for comparison as it is not corrected for the age and sex factors which obviously operate in a series of patients with cancer of the cervix uteri. The history, evaluation of the stage of involvement, and the pathologic grade of malignancy were made by independent observers and confirmed by the author.

INFLUENCE ON AGE DISTRIBUTION

It has been suggested that syphilis predisposes to the earlier appearance of cancer. The age distribution as regards extremes was the same in both groups varying from cases in the 3rd to the 8th decade. The incidence of the disease before 40 years of age shows a slight weighting on the side of the luetic, though it is not statistically significant. The average age, however, for the series of syphilitic cases was 47 years,

and for the nonsyphilitic 51.1 years. The age distribution in periods of 5-year groups is graphically shown in Fig. 1.

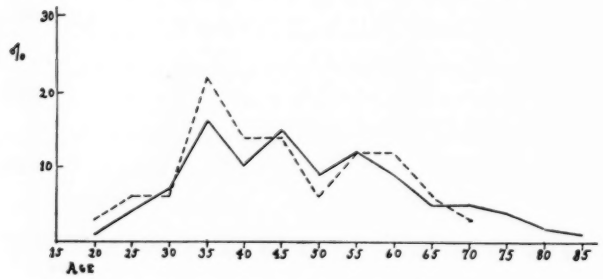

FIG. 1.—Graph showing the age distribution in 5-year periods in percentage of incidence. The dotted line represents the syphilitic cases.

grade 2 tumors are transitional cell growths between grades 1 and 3 in type. The results of grading the carcinomas in this series are shown graphically in Fig. 2. In both classes the highly malignant growths composed 20 per cent of the group. However, the grade 1 carcinomas comprised 35 per cent of the cases in the nonluetic group and only 20 per cent in the luetic group. This difference is not sufficient to be conclusive but does give an indication of a tendency in this series. Further tabulation on a larger series will be undertaken at a later date.

INFLUENCE ON CLINICAL STAGE AT FIRST EXAMINATION

At the time the positive diagnosis of cervical cancer was made each case was classified according to the

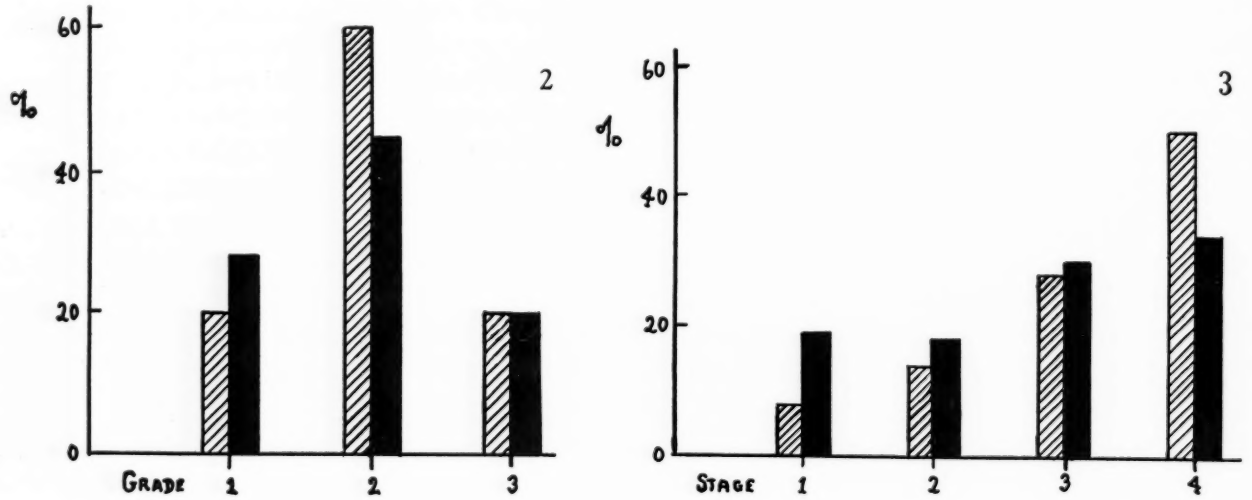

FIG. 2.—Diagram showing the percentage distribution of the pathologic grades of malignancy. The shaded area represents the syphilitic group.

FIG. 3.—Diagram showing the percentage distribution according to stage of involvement when first seen clinically. The shaded area represents the syphilitic group.

INFLUENCE ON GRADE OF MALIGNANCY

The conception of assessing the degree of (malignant) activity present in a tumor is largely credited to Broders who worked out his system of four grades in epitheliomas. Depending upon the degree of anaplasia he classifies tumors in grade 1 when the cells resemble, to a large degree, the cells from which they have originated. If the majority of the cells are poorly differentiated and abnormal in morphology they are considered to indicate a rapidly growing tumor and this is called grade 4. Grades 2 and 3 are the intervening stages. In the case of cervical epitheliomas the grading as advocated by Martzloff (3, 4) considers only three grades, as the highly differentiated type known as grade 1 in Broders' system is rarely, if ever, seen.

For our purpose we have used the Martzloff grading (3, 4). Grade 1 is composed of cells showing marked differentiation and with the presence of intercellular bridges. These may be termed the spinal cell type. The grade 3 cells are the most rapidly growing cells and are typified as the spindle cell group. The extent of clinical involvement. The importance of the degree of invasion upon the question of operability or prognosis is so well recognized as to require no further comment. The stages are numbered from 1 to 4, as follows: 1. involvement limited to cervix; 2. limited to cervix and contiguous tissue (no fixation); 3. invasion of parametrium; 4. marked fixation in pelvis or distant metastases.

In Fig. 3 the percentages in each stage of the luetic and nonluetic group are compared. It must be pointed out that 37 per cent of the nonsyphilitic group were found in stages 1 and 2 in contrast to only 22 per cent of the syphilitic group in the same stages. The importance of this difference in the prognosis of the disease is at once obvious. That this difference is not due to the time element is seen from the fact that the period of time elapsing from the appearance of the first symptom to the time of seeking medical attention

was 7.1 months in the syphilitic group and 8.4 months in the other. No significance is attached to this difference as it is probably due to several long-neglected cases in the nonsyphilitic series who entered the hospital in a moribund condition after approximately 4 years of symptoms.

INFLUENCE OF MULTIPLE PREGNANCY

It has been suggested that syphilis may exert an influence upon cancer of the cervix through the repeated injury to the cervix due to the many miscarriages which so often accompany the disease. The present series of cases was studied in this aspect and it was found that the syphilitic patients had had an average of 4.9 pregnancies while the nonsyphilitic group had an average of 4.0. This could not be considered a reliable difference as one woman in the former group had had 38 miscarriages, a number sufficient to make the difference in two averages. Twenty-one nonsyphilitic women of this series had never been pregnant, an incidence of approximately 12 per cent. This fact stresses the importance of adequate local examinations in nulliparous women who complain of bleeding or discharge from the vagina.

SUMMARY

1. Syphilitic women develop carcinoma at an average age of 47 years, as compared to 51 years in nonsyphilitic women in this series.

2. A higher per cent of nonsyphilitic cases is classified as grade 1 according to Martzloff's classifications.

3. The nonsyphilitic group showed less extensive involvement when first examined than the syphilitic group.

4. No significant difference existed in the delay in obtaining adequate therapy.

5. The influence of multiple pregnancies when the two groups are compared appears negligible.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

ANDO, T. [Pathologisches Inst. der Kaiserlichen Univ., Kyoto] EXPERIMENTELLE LEBERKARZINOMENTSTEHUNG UND GETREIDE. (IV MITTEILUNG); EXPERIMENTELLE LEBERKARZINOMENTSTEHUNG UND LEBERTRAN. [EXPERIMENTAL LIVER CARCINOGENESIS AND GRAIN; EFFECT OF COD LIVER OIL.] Gann, 35:201-204. 1941.

Rats were fed on a wheat diet for varying lengths of time after which o-aminoazotoluene (0.135%) in cod liver oil (2.6%) was added. This amount of cod liver oil appeared to be toxic and was therefore reduced to 0.054% by dissolving the cod liver oil and the o-aminoazotoluene in olive oil. This level also appeared to be toxic. The cod liver oil content was finally reduced to 0.011%. However by this time 38 of the original 60 rats had died. The livers of the remaining rats were examined for carcinomatous changes after 180, 280, and 388 days of o-aminoazotoluene feeding. According to the author cod liver oil, in the low concentration used, exerts an inhibitory effect on liver carcinoma production by o-aminoazotoluene. The constituent of the cod liver oil responsible for this effect is not known.—P. P. C.

HASHIDA, M. [Pathologisches Inst. der Kaiserlichen Univ., Kyoto] ÜBER DIE ANTIKANZEROGENITÄT VON KÜNSTLICHEN ANILINFARBSTOFFEN BEI DER EXPERIMENTELLEN HEPATOMERZEUGUNG. I MITT. [CONCERNING THE ANTICARCINOGENICITY OF SYNTHETIC ANILINE DYES IN EXPERIMENTAL HEPATOMA PRODUCTION. Gann, 35:164-166. 1941.

O-aminoazotoluene in olive oil was mixed with unpolished rice stained with the dyes, thionine (diet I), toluidine blue (diet II), and Nile blue sulfate (diet III), and fed to rats for 11 months. The rats were fed previously for one month on the same diet but without dye. During the 12-month feeding interval 12 control, 13 diet I, 9 diet II, and 12 diet III animals died with the following number of liver cancers found, respectively: 1, 1, 2, and 0. At the end of 12 months 5 animals in each group were sacrificed. The number of liver cancers found were as follows: control, 5; diet I,3; diet II, 3; and diet III, 1. The following incidence of liver cancer in animals dying after 365 days was observed: diet I, 1 died, 1 cancer; diet II, 1 died, 1 cancer; diet III, 2 died, no cancer or any sign of nodular hyperplasia of the liver. Three animals on diet II and 4 animals on diet III were still alive after 410 days. According to the author, these experiments demonstrate that certain dyestuffs may possess anticarcinogenic properties. —P. P. C.

HEIMAN, J. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] THE EFFECT OF THORIUM DIOXIDE ON NORMAL AND ESTRINIZED TUMOR-BEARING RATS. Cancer Research, 2:25-27. 1942.

In an attempt to produce a malignant transformation in benign growths, thorotrast, a radioactive substance, was injected in one series of rats with spontaneous and transplanted mammary fibroadenoma. Another similar series was given 1 mgm. of estrogenic hormone (Dimenformon benzoate) in addition. The growth rate and morphology of these benign tumors were not influenced by the injected thorotrast whether this was introduced into or near the tumors. Thorotrast injected at a site removed from the tumor was also ineffective.

In estrinized rats the glandular elements of the neoplasm were stimulated but the combined thorotrast and estrogen in no case produced any morphologic change in the tumor pointing to malignancy. At the site of thorotrast injections, there appeared swelling and induration followed after a period of 6 to 12 months by granuloma and sarcoma. No metastases were seen but infiltration of muscles, liver, spleen, and lymph nodes by thorotrast-laden cells was evident. In some animals the specific effect of estrogen and thorotrast could be noted in juxtaposition to mammary gland hyperplasia, induced thorotrast tumor, and transplanted fibroadenoma.

Thorotrast did not produce any malignant epithelial changes in benign fibroadenoma of the rat's breast. The combined action of thorotrast and estrogenic hormone was also ineffective.—Author's abstract.

ITO, S. [Aus dem Laboratorium des Gunsei-Krankenhauses] ÜBER DIE HEMMENDE WIRKUNG VON METHYLENBLAU BEI EXPERIMENTELLER HEPATOMENTSTEHUNG. II. MITTEILUNG. [CONCERNING THE INHIBITING EFFECT OF METHYLENE BLUE ON EXPERIMENTAL HEPATOMA PRODUCTION.] Gann, 35:182-185. 1941.

White rats were fed unpolished rice to which was added methylene blue(0.1%) and/or o-aminozotoluene (0.1%). The supplements were fed as follows: group I, simultaneous feeding of both methylene blue and o-aminoazotoluene; group II, o-aminoazotoluene for 200 days, then methylene blue added; group III, o-aminoazotoluene for 250 days, then methylene added. All the rats were sacrificed after 300 to 365 days and the livers examined for carcinoma. The per cent malignancy found in the different groups was as follows: group I, control, 60, methylene blue, 16.6; group II, control, 100, methylene blue, 44.4; group III, control, 77.7, methylene blue, 54.5. On the basis of these findings the author concludes that methylene blue inhibits the carcinogenicity of o-aminoazotoluene, especially if fed during the precancerous stages.—P. P. C.

KENSLER, C. J., S. O. DEXTER, and C. P. RHOADS. [Memorial Hosp., New York, N. Y.] THE INHIBITION OF A DIPHOSPHOPYRIDINE NUCLEOTIDE SYSTEM BY SPLIT PRODUCTS OF DIMETHYLAMINOAZOBENZENE. Cancer Research, 2:1-10. 1942.

A method of preparing a stable apozymase is described. One metabolite (*p*-phenylenediamine) and a probable intermediary metabolite (N,N-dimethyl-*p*-phenylenediamine) of N-dimethylaminoazobenzene, which produces hepatic cancer in rats and causes a decrease of the diphosphopyridine nucleotide content of the damaged livers, have been shown to be toxic in low concentrations to a

fermenting system in which diphosphopyridine nucleotide is the limiting factor. From a study of the metabolites and compounds related to them, it is concluded that the toxic effect is due to the formation of an intermediary oxidation product of the compound in the fermenting system. The acetyl derivatives of N,N-dimethyl-*p*-phenylenediamine and *p*-phenylenediamine are not toxic to the system. Alloxan has also been found to be highly toxic to the system.

These experiments indicate that the inhibition of this system both by the Wurster type compounds and alloxan is a competitive one in which the substance competes with diphosphopyridine nucleotide for an enzyme active in fermentation. The evidence suggests that the triosephosphate dehydrogenase is the enzyme which is inactivated by these compounds. The apparent correlation between toxicity in this system of the diamine split products of methyl derivatives of aminoazobenzene and the carcinogenic potency for the rat liver of the parent molecule is discussed.—Author's summary.

MARTIN, R. H. [The Roy. Cancer Hosp. (Free), London] POLYCYCLIC AROMATIC HYDROCARBONS. PART XXVIII. DIBENZFLUORENES. J. Chem. Soc., 679-685. 1941.

The molecular structure of the carcinogenic hydrocarbon 1,2,7,8-dibenzfluorene has been confirmed by degradation to 2,2′-dinaphthyl, and by the synthesis of 2,3,6,7- and 1,2,6,7-dibenzfluorenones, which were found to differ from the ketone formed by oxidation of 1,2,7,8-dibenzfluorene. These three compounds represent all the possible dibenzfluorenones related to 2,2′-dinaphthyl. 3,4,5,6-Dibenzfluorene has been synthesized and is under test for carcinogenic action.—E. L. K.

NAKAHARA, W., and K. MORI. [Labs. of the Japanese Foundation for Cancer Research, Tokyo] EXPERIMENTAL PRODUCTION OF LIVER CIRRHOSIS BY FURFURAL FEEDING. Gann, 35:210-233. 1941.

In view of the prevalence of liver cancer in Japan and other oriental countries, sake, the Japanese rice wine, was thoroughly investigated as a possible etiological factor. The following known components of sake were fed to about 700 rats along with polished rice for 300 days: evaporated sake (containing carbohydrates, amines, amino acids, nonvolatile acids, esters, etc.), acetaldehyde, furfural, methyl, ethyl, propyl and butyl alcohols, methyl-ethyl ketone, and glycerol. Of these compounds furfural alone produced a significant pathological change, viz., cirrhosis of the liver. The furfural was fed initially at a level of 10 to 20 ml. per kg. of rice which was gradually raised to 50 ml. per kg. It was found that doses of furfural capable of inducing cirrhosis in 100 days invariably resulted in the death of the rats in about 200 days. Smaller doses permitted longer survival but delayed production of cirrhosis. Interruption of furfural feeding after 142 days allowed survival well beyond 200 days, but nevertheless all the animals showed advanced liver cirrhosis when examined after death. The cirrhotic changes appeared to be initiated by a primary productive interstitial process, without notable necrosis of liver cells, and were characterized by a prominent annular appearance and proliferation of pseudo-bile ducts. While the histological picture resembled that seen in the precancerous stages of livers from rats fed azo dyes, no case of liver cancer was observed following furfural feeding. The spleen and other organs showed no pathological changes.—P. P. C.

NELSON, A. A., O. G. FITZHUGH, H. J. MORRIS, and H. O. CALVERY. [Division of Pharmacology, Food and Drug Administration, Washington, D. C.] NEUROFIBROMAS OF RAT EARS PRODUCED BY PROLONGED FEEDING OF ERGOT. Cancer Research, 2:11-15. 1942.

Histologically typical neurofibromas have been produced on the ears, and on the ears only, of a high percentage of rats by prolonged feeding of 5% of crude ergot in the diet. The tumors have occurred less frequently on a level of 2% of crude ergot and not at all on a level of 1%. A low protein diet somewhat favors the production of tumors.

The neurofibromas have been made to regress markedly by withholding ergot, and then to reappear by refeeding. After about 6 months without feeding of ergot, some tumors which have markedly regressed will spontaneously grow again; however, this is practically at the end of the life span of the animals.

Two other lesions, a renal medullary necrosis and calcification, and enlargement of the ovaries, are frequently caused by feeding of ergot. No cutaneous gangrene and no vascular lesions attributable to ergot have been observed, probably because the dosage in terms of alkaloids has been much too low.

The exact constituent of the crude ergot responsible for the tumor production is not known.—Authors' abstract.

NOTIK, L. V. [I. P. Pavlov I Med. Inst., Leningrad] THE EFFECT OF THE NERVOUS SYSTEM UPON THE INITIATION OF EXPERIMENTAL CANCER. Bull. biol. et méd. expér. URSS., 5-6:507-509. 1940.

The lower part of the back of mice was painted for 6 months with tar or for 3 months with 3,4-benzpyrene. At the time of appearance of papillomas, the right sciatic nerve was cut at about the level of the middle of the thigh, and its central end punctured by a pin moistened with formal or croton oil. Six series of experiments were made, comprising 114 mice.

In the series using tar, degeneration of papillomas occurred in 19 experimental animals as compared with 2 in the control groups; while 8 carcinomas developed in the experimental groups as compared with 18 among the controls. Thirteen trophic ulcers also appeared among the experimental mice.

In the series in which benzpyrene was used degeneration of papillomas was again much more frequent among experimental mice than in the controls, while a smaller number of carcinomas had developed (1.5 months). Prolonged observation on benzpyrene-treated mice showed that some of the animals operated upon ultimately developed cancer, and the ulcers gradually healed.—M. B.

POLLIA, J. A. [The Frank H. Boyer Foundation for Med. Research, Los Angeles, Calif.] INVESTIGATIONS ON THE POSSIBLE CARCINOGENIC EFFECT OF ANTHRACENE AND CHRYSENE AND SOME OF THEIR COMPOUNDS. II. THE EFFECT OF SUBCUTANEOUS INJECTION IN RATS. J. Indust. Hyg. & Toxicol., 23:449-453. 1941.

Three paints, green anthracene water-color, blue chrysene water-color, and blue chrysene oil-color, and their

bases, anthracene and chrysene, were administered weekly by subcutaneous injection to Wistar rats. The total number of injections varied from 4 to 11. These paints showed no carcinogenic activity when compared with 1,2,5,6-dibenzanthracene. The results parallel those previously obtained with skin painting in mice.—F. L. H.

PULLINGER, B. D. [The Imperial Cancer Research Fund, London] CORRELATION BETWEEN CARCINOGENIC POTENCY AND THE FIRST SKIN REACTION TO CERTAIN HYDROCARBONS. J. Path & Bact., 53:287-288. 1941.

In an earlier paper Pullinger (J. Path. & Bact., **50**:463. 1940.) described a characteristic reaction (swelling of epithelial cells and nuclei, multiplication at first by direct and later by indirect division) following application of the more rapidly acting carcinogenic hydrocarbons to the skin of the mouse. The present paper compares in the case of 8 compounds (5,9,10-trimethyl-1,2-benzanthracene, 9,10-dimethyl-1,2-benzanthracene, methylcholanthrene, 3,4-benzpyrene, 3,4,5,6-dibenzcarbazole, 6,9,10-trimethyl-1,2-benzanthracene, 2-methyl-3,4-benzphenanthrene, 5,6,9,10-tetramethyl-1,2-benzanthracene) the minimum effective dose (a) which produces this reaction with the latent period of tumor production (b), and the percentage production of tumors (c). The shorter the average latent period for carcinogenesis the lower was the dose required to cause the early characteristic response. There is a less close relationship to the percentage of tumor-bearing animals. The quotient $\frac{c}{b}$ was used by Iball. (Am. J. Cancer, **35**:188. 1939.) as a measure of carcinogenic power. A dose about 6 times greater than that required to produce the hyperplastic reaction causes in some areas thinning and stretching of epithelial cells so that they appear atrophic. The noncarcinogenic 1,2′-azonaphthalene (Cook, J. W., C. L. Hewett, E. L. Kennaway, and N. M. Kennaway. Am. J. Cancer, **50**:62. 1940.) in the highest dose used does not induce the hyperplastic reaction.—E. L. K.

ROFFO, A. H. [Inst. de med. exper. para el estud y trat. d. cáncer, Buenos Aires] ALQUITRAN CANCERíGENO DE YERBA MATE. [CARCINOGENIC TAR FROM MATE WEED.] Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 56:5-18. 1941.

The human consumption of mate weed (*Ilex paraguayensis*) in infusion is 6 and 24 times as large, in Argentina, as that of tea and coffee, respectively. In Brazil it is ingested at the ratio of 10 kg. per inhabitant. The lipoid content is 9.80 gm. per kg. as compared to 7.61 for coffee and 1.87 for tea. By distillation at 350° C. a thick, black alkaline product is obtained similar to the tars obtained from coffee and tea and, as in these two products, devoid of caffeine. The yield is 180 cc. per kg. of weed. The application every other day of this tar to the internal part of both ears of 10 rabbits induced malignancy in every case following the usual sequence hyperkeratosis-papilloma-epithelioma. The earliest lesions appeared from 87 to 355 days after the beginning of the treatment. Since extraction of the weed with boiling water does not involve the passage into the infusion of the water-insoluble carcinogen the author does not think that consumption of the weed infusion is of any danger.—M. D-R.

ROFFO, A. H. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires] CANCERIZACIÓN GÁSTRICA POR INGESTIÓN DE ALQUITRÁN TABÁQUICO. [GASTRIC CANCERIZATION BY INGESTION OF TOBACCO TAR] Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 56:39-69. 1941.

As previously reported the author has obtained a tar tobacco endowed with strong carcinogenic power when applied to the ears of rabbits. The active product is a hydrocarbon showing the spectrographic characteristics and the fluorescence of 1,2-benzpyrene. From a kilo of tobacco one can obtain 60 to 120 cc. of tar and 0.13 cc. of this product is well supported by rats after ingestion. A 1% suspension of this tar was mixed with the food (a mixture of milk and bread) at the ratio of 0.15 cc. per 25 cc. An unspecified number of rats were given an unspecified amount of tar and 50% of these animals developed hemorraghic ulceration which evolved into ulcus rodens and "real cancer" in a period ranging from 5 to 30 months. The summarized stories of 20 of these animals, 3 color plates, and 21 photomicrographs are appended.—M. D-R.

WHITE, P. R., and A. C. BRAUN. [Rockefeller Inst. for Med. Research, Princeton, N. J.] CROWN GALL PRODUCTION BY BACTERIA-FREE TUMOR TISSUES. Science, 94:239-241. 1941.

Although crown gall can readily be produced in plants by inoculation of the host with *Phytomonas tumefaciens*, the earlier work of Smith indicating that true secondary tumors (in sunflower plants) may develop has only recently been confirmed.

Tissue fragments were removed from the interior of such secondary growths and grown in White's nutrient medium. Of 107 original isolations, about 6% were contaminated with bacteria and molds. Of 50 control cultures, 14% showed such contamination.

About half the cultures of tissue from large secondary tumors grew successfully *in vitro*. One culture had, at the time of writing, been maintained through 13 successive passages (482 pieces) with large increase in volume. Although in constant contact with a medium that supports luxuriant growth of *Phytomonas tumefaciens* not one culture developed bacterial growth; and attempts to isolate bacteria from these cultures by various methods were consistently negative. When such cultures were ground and the paste injected into sunflower or tomato plants no galls were produced, as is the case when a similarly prepared paste from young primary tumors is used.

After 5 successive passages *in vitro*, 10 tumor cultures were grafted back into sunflower plants; at the end of 7 weeks, half of them produced typical crown gall tumors. Cultures from the 6th and 10th passage behaved similarly.

These results furnish evidence that these tumor tissues proliferate without continued stimulation from crown gall bacteria. Their capacity for autonomous growth appears established.

Gross and microscopic characteristics of cultures of tumor and normal tissues and of tumor grafts are described.—M. B.

BIOCHEMISTRY AND NUTRITION—CHEMOTHERAPY

BADGER, G. M. [The Roy. Cancer Hosp. (Free), London] THE SYNTHESIS OF GROWTH-INHIBITORY POLYCYCLIC COMPOUNDS. PART III. J. Chem. Soc., 535-538. 1941.

The preparation of 9-methyl-1,2-benzfluorene, α-1-naphthyl-β-2-naphthylethylene, and some phenyl-naphthylethylenes, is described. All three classes of compound are related to known potent tumor-inhibiting agents and have been prepared for biological test.—E. L. K.

FUKUOKA, F. [Labs. of the Japanese Foundation for Cancer Research, Tokyo] EFFECT OF GIBBERELLIN ON TISSUE CULTURE. Gann, 35:205-207. 1941.

Gibberellin is the substance produced by the fungus *Gibberella fujikuroi,* and has been shown to be responsible for the overgrowth of infected rice plants without grain production. Since this substance has growth-accelerating properties for plant cells, the author studied its effect on tissue cultures. No evidence was found for any influence of gibberellin on the growth of cultures of embryonic chicken heart.—P. P. C.

LAURENCE, W. L. INDUCED BIOTIN DEFICIENCY AS A POSSIBLE EXPLANATION OF OBSERVED SPONTANEOUS RECESSIONS IN MALIGNANCY. Science, 94:88-89. 1941.

It has been established that biotin (coenzyme R, vitamin H) is essential for the vital functions of many forms of plant and animal life. It has also been shown that commercial or fresh egg white inactivates biotin *in vitro* and produces "egg white injury" *in vivo,* due to a constituent called avidin which, because of its biotin-binding capacity causes a deficiency in this substance. In addition, studies on the content of tumors and other tissues showed the high biotin level of the tumor deviating from the normal low adult values in the same direction as the corresponding embryo tissues. The writer offers the following hypotheses in explaining reported spontaneous recessions of human malignant tumors, especially when connected with acute infections:

1. Both the malignant cells and the micro-organisms associated with the observed cases of spontaneous recession require excess biotin for their metabolic activities. 2. Spontaneous recessions could be explained as the direct result of biotin deficiency brought about by the avidin-like action of the micro-organisms depriving the malignant cells of a factor vital for existence. 3. Raw egg white, or avidin, because of their ability to deprive pathogenic bacteria and malignant cells of a life-essential factor suggest themselves as new therapeutic agents in conditions due to the presence of these entities. An induced biotin deficiency could be controlled by administration of definite amounts of biotin.—M. B.

LEUTSCHER, J. A. [Harvard Med. Sch., Boston, Mass.] ELECTROPHORETIC ANALYSIS OF THE PROTEINS OF PLASMA AND SEROUS EFFUSIONS. J. Clin. Investigation, 20:99-106. 1941.

By means of the Tiselius electrophoretic technic, human plasma and serous effusions have been analyzed in patients with the following diseases: cirrhosis, cardiac failure, terminal glomerulonephritis with heart failure, lobar pneumonia, tuberculosis, Hodgkin's disease, and carcinoma. The proteins of the serous effusions show the same electrophoretic fractions (albumin, alpha-globulin, beta-globulin, gamma-globulin, and fibrinogen) as the plasma proteins. However the amounts of the various components in the fluid are determined by the composition of the plasma protein and by the factors producing the effusion. The fluids occurring in connection with tumors are extremely variable. When there is hemorrhage or oozing of serum into a serous cavity from invasion of the lining, the blood may be normal and the fluid is nearly identical with blood in terms of protein content. When the tumor gives rise to localized obstruction of the veins and lymphatics, the fluid is more dilute resembling that of cardiac failure.—J. L. M.

LEWISOHN, R., C. LEUCHTENBERGER, R. LEUCHTENBERGER, D. LASZLO, and K. BLOCH. [Mount Sinai Hosp., New York, N. Y.] PREVENTION OF TUMOR GROWTH (CARCINOMA 2163) BY INTRAVENOUS INJECTIONS OF YEAST AND VITAMINS. Science, 94:70-71. 1941.

The tumor used was a mammary adenocarcinoma (Ca 2136) which arose in the inbred French strain R III and is transplantable 95 to 100% in this strain. A number of vitamins (pantothenic acid, riboflavin, and thiamin) and a yeast extract were given singly or in various combinations. The vitamins had very little or no effect on growth of the transplants. Yeast alone prevented tumor growth in about 20%. This tumor-preventing effect of yeast was increased by adding pantothenic acid (nontakes 47%) or riboflavin (nontakes 62%) to the yeast extract. Addition of thiamin to the yeast extract did not augment the tumor-preventing action.—M. B.

SALZBURG, P., and H. KABAT. [Univ. of Minnesota, Minneapolis, Minn.] DIFFERENTIAL SENSITIVITY OF SARCOMA AND NORMAL TISSUES TO TEMPORARY ARREST OF CIRCULATION. Arch. Surg., 42:917-928. 1941.

The authors produced sarcomas in the hindlegs of 3-month-old rats by injecting methylcholanthrene subcutaneously. Several months later, when tumors became palpable, they interrupted the blood flow with an inflatable cuff applied to the thigh. They found that complete arrest of the circulation for 6 to 7.5 hours causes extensive central necrosis of tumor tissue, but some viable tumor cells always persist, usually in the periphery. Normal tissues, on the other hand, are more resistant to arrest of blood flow. They conclude, therefore, that arresting the circulation does not cure malignant neoplastic tissues.—G. De B.

STRONG, L. C., and F. H. J. FIGGE. [Yale Univ. Sch. of Med., New Haven, Conn., and Univ. of Maryland Med. School, Baltimore, Md.] FLUORESCENCE OF HARDERIAN GLANDS IN MICE OF CANCER-SUSCEPTIBLE AND CANCER-RESISTANT STRAINS. Science, 94:331. 1941.

Red fluorescence to ultraviolet light (G.E. B-H^4) was observed in the exposed Harderian glands of mice of various strains. Considerable strain variation was found. In mice older than 300 days, the JK(cancer-resistant) strain showed least and the C3H(cancer-susceptible) strain most fluorescence. Mice of inbred strains exhibited greater constancy than those of heterozygous strains (NH). An age variation was also noted. Fluorescence was absent before 14 days, was high in early maturity, decreased with advancing age, and was completely absent in old mice (JK strain). Such decrease in fluorescence with old age was not observed in C3H mice.

Red fluorescence of the Harderian glands is held to be due to the presence of porphyrins. Some physiological implications of this phenomenom are briefly discussed. —M. B.

Hormones

BISCHOFF, F., M. L. LONG, J. J. RUPP, and G. J. CLARKE. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] CARCINOGENIC EFFECT OF ESTRADIOL AND OF THEELIN IN MARSH-BUFFALO MICE. Cancer Research, 2:52-55. 1942.

Mice of the Marsh-Buffalo strain receiving 0.08 cc. weekly injections of sesame oil showed a lower tumor rate than that of untreated mice. A 12 to 13% incidence of early death indicated a toxic effect. In groups of 40 mice, 35% of theelin-dosed mice and 20% of estradiol-dosed mice died within a 7-month period. Approximately 4 mgm. of estrogen was administered per mouse in a 6-month period. Estradiol was not more toxic in ovariectomized mice than in intact mice and not more toxic than theelin (on a weight basis). The incidence of lymphosarcoma was definitely increased by estradiol dosage in both intact and ovariectomized mice. The increase in the theelin-dosed mice was doubtfully significant. In the intact mouse, the sublethal doses of theelin and estradiol increased the tumor rate of breast tumor formation if the incidence is calculated on the basis of surviving mice. In the ovariectomized mouse, estradiol maintained the rate at approximately that of the controls.—Authors' abstract.

GROSS, L. [Inst. for Med. Research, Christ Hosp., Cincinnati, O.] INFLUENCE OF SEX ON THE EVOLUTION OF A TRANSPLANTABLE MOUSE SARCOMA. Proc. Soc. Exper. Biol. & Med. 47:273-276. 1941.

A suspension of mouse sarcoma S37 in 0.85% saline was used as the inoculum. Injections were made intradermally into the freshly shaved skin in the middle of the back of adult, white mice, of the same stock strain (not specified). When small doses were employed, the incidence of takes was higher in the males (68%) than in the females (43%). When heavier doses were used there was no difference in takes between the sexes. In some instances, the cutaneous tumors increased in size and killed their hosts within 20 to 80 days after inoculation. The average survival time of the males was shorter than that of the females. Spontaneous disappearance of some tumors within 15 to 30 days after inoculation was observed. The incidence of such spontaneous regressions was higher in females (54%) than in males (16%), when either small or medium-sized doses of tumor cells were inoculated. This sex difference in incidence of tumor regression was less striking when high doses of tumor cells were used.—M. B.

SMITH, D. L., J. A. WELLS, and F. E. D'AMOUR. [Univ. of Denver, Colo.] THE RELATIONSHIP OF THE ENDOCRINE SYSTEM TO CARCINOGENESIS. Cancer Research, 2:40-44. 1942.

In this study the possible influence of various hormones upon the rate of development of chemically induced tumors was investigated. Methylcholanthrene in paraffin solution was injected into 5 sites, the total dose being 10 mgm. The influence of each hormone was determined by using two contrasting groups, one consisting of animals in which the gland concerned had been extirpated, the other including animals to which excess amounts of the hormone were administered. The study includes 25 experimental groups, each group consisting usually of 10 animals. Variations in the time elapsing between injection of the carcinogen and the appearance of a tumor of standard size were considered the criterion of influence of the hormonal condition.

In general it was found that deprivation or excess of most hormones had little effect upon the development of the tumors. The average latent period for normal rats was found to be 106 days; the most rapidly developing tumors, which occured in animals receiving chorionic gonadotropins, was 79 days, and the most slowly developing tumors, 122 days, occured in animals receiving adrenal cortical extract. Only in the groups mentioned were the variations of statistical significance. It is noteworthy that neither deprivation nor excess of the sex hormones had any significant influence on the rate of development.—Authors' abstract.

LIPSCHÜTZ, A., R. THIBAUT, and L. VARGAS, JR. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] THE FIBROMATOGENIC ACTION OF SPECIFIC URINARY ESTROGENS (METAHORMONES) IN THE GUINEA PIG. Cancer Research, 1:45-51. 1941.

The ovarian estrogens, estrone and estradiol, and the specific urinary estrogens, estriol and equilenin, were implanted in the form of pellets subcutaneously into castrated female guinea pigs. Observations were made upon the rates of absorption and the comparative effects of these estrogens upon uterine weights and their fibromatogenic activities.

Specific urinary estrogens, such as estriol and equilenin, elicited abdominal serosal fibroids similar to those following subcutaneous implantation of pellets of estradiol and estrone. The fibromatogenic action of estriol was less than that of estradiol and estrone. The fibromatogenic action of equilenin was insignificant.

The stronger action of estradiol and estrone as compared with that of the specific urinary estrogens was not due to absorption of larger quantities of the ovarian estrogens. On the contrary, estrone was absorbed more slowly and was more effective than twice the quantities of absorbed estriol and equilenin.

Estriol produced increase of uterine weight almost equal to that produced by estradiol and estrone; the increase with equilenin was smaller. The differences between the hysterotropic actions was less significant than the differences between the fibromatogenic effects.

When a steady flow of estrogen is established from a subcutaneously implanted pellet, abdominal fibroids can be elicited with about 5 μgm. of estrone absorbed per day, as compared with 400 μgm. injected thrice weekly, in the course of 4 months. This difference is explained by the assumption that the tumorigenic faculty of estrogens is dependent upon a continuous action upon the effector tissues.

Under the same experimental conditions which provided for continuous action, quantities of equilenin, two or three times as large as the effective amounts of estrone, had no fibromatogenic action or only an insignificant effect.—Authors' summary.

Genetics

BLUMENTHAL, H. T. [Washington Univ. Sch. of Med., St. Louis, Mo.] HOMOIOTRANSPLANTATION OF SPON-

TANEOUS TUMORS INTO MICE BEARING SPONTANEOUS TUMORS. Cancer Research, 2:56-58. 1942.

In these experiments the original observations of Loeb and Fleisher that mice which are the bearers of spontaneous tumors offer a better soil for the growth of transplanted spontaneous tumors than normal mice is confirmed. In 52 mice which were the bearers of spontaneous tumors a transplanted spontaneous tumor from a mouse of a different strain grew progressively in 11 instances (21.2%), while in 52 normal mice of corresponding age and strain no successful transplantations of the same spontaneous tumors from different strains were observed. The successful growth of transplanted tumors was found only in mice which were below the age of 12 months. This suggests that the age of the host may be one of the factors which determines the success of the transplantations. In 14 additional mice bearing spontaneous tumors, the inoculated tumor grew temporarily for a period of approximately 7 to 10 days and then gradually retrogressed, whereas complete regression in controls always occurred within 4 to 6 days.—Author's Abstract.

SNELL, G. D., Editor. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] MOUSE GENETICS NEWS, NO. 1, 1-18. 1941.

A mimeographed publication prepared at the Roscoe B. Jackson Memorial Laboratory with the collaboration of investigators using mice in biological research. This issue contains: (1) the rules adopted by the International Committee on Mouse Genetics Nomenclature for assigning symbols to mutations, (2) a list of mutant genes of the mouse with the symbols recommended by the International Committee on Mouse Genetics Nomenclature, (3) a list of 69 inbred strains, giving inbreeding, fertility, genetics, origin, characteristics, and references, (4) a list of the contributing laboratories with the stocks maintained by each. In this issue only American and Canadian laboratories are included.—Editor's summary.

Physical Factors

SANO, M. E., and L. W. SMITH. [Temple Univ. Sch. of Med., Philadelphia, Pa.] THE BEHAVIOR OF TUMOR CELLS IN TISSUE CULTURE SUBJECTED TO REDUCED TEMPERATURES. Cancer Research, 2:32-39. 1942.

The behavior of tumor tissue from cases of reticulum cell sarcoma, colloid carcinoma, acute lymphoid leukemia, Hodgkin's disease, and experimental breast cancer in mice (Lankenau C57 strain) under varying temperatures ranging from 0-37° C. were studied *in vitro*. A critical level of around 22-24° C. checked mitosis through interference with nuclear division. It was shown further that there is some variation in individual cell resistance to these lower temperatures and that the time interval as well as the temperature must be taken into consideration in any experimental studies designed to demonstrate the variation of cell response. The paper compares the behavior of the several tumors clinically with their behavior as grown *in vitro*.—Authors' abstract.

Radiation

HALBERSTAEDTER, L., G. GOLDHABER, and L. DOLJANSKI. [Hebrew Univ., Jerusalem] COMPARATIVE STUDIES ON THE RADIOSENSITIVITY OF NORMAL AND MALIGNANT CELLS IN CULTURE. I. THE EFFECT OF X-RAYS ON CELL OUTGROWTH IN CULTURES OF NORMAL RAT FIBROBLASTS AND RAT BENZPYRENE SARCOMA. Cancer Research, 2:28-31. 1942.

The cultures of fibroblasts were obtained from rat embryos, and were in the 2nd or 3rd passage when used in the irradiation experiments. The cultures of sarcoma cells were from a tumor induced by benzpyrene in the rat. Tissue cultures were made according to the cover slip method of Carrel. The culture medium was composed of chicken plasma and diluted chicken embryo extract in equal proportions. Irradiation was supplied by a demountable x-ray tube operated at 35 kv., 20 ma., with copper anticathode and a window of aluminum foil 30 μ thick. The x-ray intensity at the distance of the irradiated object was 90,000 r/min. The rays which penetrated the culture were mainly copper-k-rays. Outgrowth from explants and appearances of cells were measured and observed after irradiation of the cultures at different dosages from 10,000 r to 200,000 r.

The minimum x-ray dose which totally suppresses cell outgrowth in tissue cultures was found to be the same for both normal rat fibroblasts and rat sarcoma cells. This dose was 200,000 r.—S. B-J.

SUGIURA, K. [Memorial Hosp., New York, N. Y.] THE EFFECT OF RADIOACTIVE PHOSPHORUS ON THE VIABILITY OF MOUSE SARCOMA 180. Cancer Research, 2:19-24. 1942.

An investigation has been made of the effect of immersing fragments of mouse sarcoma 180 in radioactive phosphorus solution prior to transplantation.

The growth capacity of mouse sarcoma 180 was unaffected when tumor fragments were immersed for 24 hours at 4-5° C. in P^{32} solution having an activity of 50 μc./cc. Immersion in P^{32} solution of 75 μc./cc. resulted in about 25 per cent inhibition. Marked inhibition and retardation of growth were caused by exposure to P^{32} solution of 100 and 125 μc./cc. (about 50 and 75 per cent inhibition, respectively). The viability of the mouse sarcoma 180 was destroyed by immersion in P^{32} solution of 150 μc./cc.

The lethal effect produced by beta rays of P^{32} on the tumor was compared with that produced by roentgen rays at 200 kv. since it is possible to calculate the beta-ray emission of the radioactive phosphorus in terms of "equivalent roentgens." Under the stated conditions of the experiment it was found that the transplantability of mouse sarcoma 180 was not altered appreciably by irradiation of tumor fragments with 2,500 equivalent roentgens. An exposure of 3,500 equivalent roentgens gave about 20 per cent inhibition. Marked inhibition was caused by an exposure of 4,500 and 5,500 equivalent roentgens (about 50 and 80% inhibition, respectively). The viability of the tumor was completely destroyed by an exposure of 6,500 equivalent roentgens.—Author's summary.

TUTTLE, L. W., L. A. ERF, and J. H. LAWRENCE. [Univ. of California, Berkeley, Calif.] STUDIES ON NEOPLASMS WITH THE AID OF RADIOACTIVE PHOSPHORUS. II. THE PHOSPHORUS METABOLISM OF THE NUCLEOPROTEIN, PHOSPHOLIPID, AND ACID SOLUBLE FRACTIONS

OF NORMAL AND LEUKEMIC MICE. J. Clin. Investigation, 20:57-61. 1941.

The manner in which a single tracer dose of radioactive phosphorus is handled over a period of several days by the tissues of 50 leukemic mice and 50 normal mice has been determined. Leukemic infiltration is accompanied by a seven-fold increase in the uptake and retention of radioactive phosphorus by the nucleoprotein and acid soluble fractions of mouse liver, spleen, and lymph nodes. The phospholipid metabolism of spleen and lymph nodes is affected to only a limited extent by leukemic infiltration, while that of liver is depressed only during the first day after the administration of the radioactive phosphorus.—J. L. M.

TUTTLE, L. W., L. A. ERF, and J. H. LAWRENCE. [Univ. of California. Berkeley, Calif.] STUDIES ON NEOPLASMS WITH THE AID OF RADIOACTIVE PHOSPHORUS. III. THE PHOSPHORUS METABOLISM OF THE PHOSPHOLIPID, ACID-SOLUBLE AND NUCLEOPROTEIN FRACTIONS OF VARIOUS TISSUES OF NORMAL AND LEUKEMIC MICE FOLLOWING THE ADMINISTRATION OF "TRACER" AND "THERAPEUTIC" DOSES OF RADIOPHOSPHORUS. J. Clin. Investigation, 20:577-581. 1941.

"Tracer" doses of radioactive phosphorus, P^{32}, are small amounts which are conceivably insufficient to cause significant changes in the metabolism of the cells in which they are retained, while "therapeutic" doses are large amounts of P^{32} which significantly alter the metabolism of cells because of the quantity of beta-radiation emitted.

Less P^{32} was retained in the phospholipid, acid-soluble, and nucleoprotein fractions of spleen, liver, lymph nodes, and carcasses both of normal mice and mice with lymphoma after intraperitoneal administration of therapeutic doses of P^{32} than when tracer doses were given. The amounts are expressed as % of the dose of P^{32} administered per gm. of wet weight of fresh tissue. There was no difference in radiosensitivity of the metabolic processes studied in the normal animals when compared with those of the leukemic animals.

The authors believe that this technic could be used as a method to compare the radiosensitivity of various types of cellular metabolism both in normal and in neoplastic tissues, and it may prove to be a valuable method of comparing the effects on these tissues of different types of radiation such as x-radiation or neutron radiation.—J. L. M.

Cytology

DOLJANSKI, L., and E. TENENBAUM. [The Hebrew Univ., Jerusalem] CELLULAR COMPOSITION OF PURE ROUS SARCOMA CULTURES IN VITRO. Proc. Soc., Exper. Biol. & Med., 47:239-241. 1941.

The pure Rous sarcoma culture consists of 2 cell types, spindle cells and round cells. The spindle cell structure corresponds to that of the normal fibrocyte. The round cell has a basophilic, homogeneous structure of finely granular cytoplasm and large excentric nucleus which has a thick membrane, 1 to 2 large nucleoli, and finely divided chromatin.

The fibroblast-like cell and the round cell are but different aspects of the same cell. The transformation of the spindle type into the round cell takes place by contraction of the former; this process is reversible. Proteolytic liquefaction of the growth medium also results in spindle cells assuming the round form. By adding various amounts of embryonic extract, the proteolytic capacity of the sarcoma cell and hence its shape can be influenced experimentally.

The pure Rous sarcoma consists of but one cell type: a mesenchyme cell which appears at times as a spindle, at others as a basophilic round cell, and is the actual carrier of the Rous sarcoma agent.

These studies were made on living cultures and on preparations fixed in Carnoy or Zenker formol and stained with Giemsa.—M. B.

GUYER, M. F., and P. E. CLAUS. [Univ. of Wisconsin, Madison, Wis.] INCREASED VISCOSITY OF CELLS OF INDUCED TUMORS. Cancer Research, 1:16-18. 1941.

The failure of either cell body or nucleus of tumor cells induced by butter yellow (*p*-dimethylaminoazobenzene) to stratify when centrifuged at high speed along with adjacent normal liver cells which do stratify markedly, is evidently due to increased viscosity of cellular contents. The condition comes on gradually as the abnormal regions in the liver become more and more tumor-like in appearance. It is suggested that the increasing lactic acid output of the tumor cell may be the cause of its enhanced viscosity.—Authors' abstract.

TENENBAUM, E., and L. DOLJANSKI. [The Hebrew Univ., Jerusalem] NUCLEAR CHANGES IN ROUS SARCOMA CELLS CULTIVATED IN VITRO. Proc. Soc. Exper. Biol. & Med., 47:236-239. 1941.

The nuclear ground substance in Rous sarcoma cells often appears as if precipitated. In some cells particulate masses become visible. At first they are equally distributed, but later gather in the center of the nucleus, leaving a clear zone between the particulate aggregates and the nuclear membrane. At times very fine threads are seen to cross this zone radially. This granular material is usually acidophilic. The nucleoli may become round or rod-shaped and appear as large, compact, deeply stained bodies. Not infrequently the nucleoli become fragmented, and sometimes contain vacuoles. At times in hypertrophied basophilic round cells the nuclei contain sharply defined, amorphous, hyaline inclusions lying between the nuclear membrane and a nucleolus. The nuclei of the Rous sarcoma cells show a tendency to fragmentation. These studies were made on cultures fixed in Carnoy and stained with Giemsa.—M. B.

Miscellaneous

BRYAN, C. C., and S. WARREN. [New England Deaconess Hosp., Boston, Mass.] FUNCTIONAL ACTIVITY OF SMOOTH MUSCLE TUMORS OF THE UTERUS. Proc. Soc. Exper. Biol. & Med., 47:356-358. 1941.

Sixteen uteri containing leiomyomas were obtained immediately after excision. Within a few minutes muscle strips about 2.5 cm. in length and 0.5 cm. in diameter were cut from the tumor and similar control strips cut from the uterine wall. These were immersed in oxygenated Ringer's solution at 38° C. In each experiment a strip of leiomyoma and one of uterine muscle were so arranged as to give recordings at different levels on the

same graph. The stimulating agents, added directly to 250 cc. of the Ringer's solution were (1) pituitrin, 0.25 to 0.5 cc. (2) histamine, 0.5 cc. of a 1:1000 solution, and (3) 5 drops of ergot.

Uterine muscle reacted more constantly to pituitrin, while muscle from leiomyomas was more sensitive to histamine although in each case some stimulation by the other substances was also obtained. Ergot failed to elicit response in either type of muscle.—M. B.

COMPARATIVE ONCOLOGY

BRIGGS, R. W. [McGill Univ., Montreal, Canada] THE DEVELOPMENT OF ABNORMAL GROWTHS IN RANA PIPIENS EMBRYOS FOLLOWING DELAYED FERTILIZATION. Anat. Rec., 81:121-135. 1941.

When the eggs of the frog (*Rana pipiens*) were allowed to remain in the uterus for periods ranging from 3 to 5 days before they were fertilized, the resulting embryos developed the following types of abnormal growths: (1) A cauliflower-like ectodermal growth 6 to 10 cells in thickness which appeared first in the neurula stages and was definitely benign. (2) A nodular growth of the ectoderm made up of a small number of much elongated cells. This growth developed on neurulae and was also benign. (3) A nonvesiculated papilloma-like ectodermal growth 6 to 10 cells thick which developed first in 4 to 8 mm. embryos. In 15% of the cases observed it spread progressively over the surface of the host. It retarded the growth of the host, and death ensued about 3 days after the first appearance of the growth. (4) Smooth-surfaced enlargements in the trunk regions. These were found to be made up of somatic mesoderm or simply to be hernias of a fold of the gut through both splanchnic and somatic mesoderm. Both types of enlargement appeared to be benign.

In addition to these abnormal tissue growths following delayed fertilization, a wide variety of gross structural abnormalities was also observed.—J. L. M.

Clinical and Pathological Reports

THERAPY—GENERAL

HODES, P. J., and R. S. THORNER. [Philadelphia, Pa.] COBRA VENOM IN THE RELIEF OF PAIN DUE TO CANCER. Am. J. Roentgenol., 45:866-870. 1941.

The chemistry and pharmacology of cobra venom are briefly reviewed and its value assessed in the relief of pain in cancer. The dose generally used is equivalent to 0.05 to 0.15 mgm. of dried venom injected intramuscularly each day until pain is relieved. Temporary exacerbation of pain may occur as well as abdominal cramps and diarrhea but these complications are not severe when moderate doses are used. Animal experiments suggest that cobra venom should not be used in the presence of severe liver or kidney damage. The authors administered cobra venom to 21 patients with pain caused by cancer. The result was excellent in 11, 6 had partial relief, and 4 were not benefited. The results were thus encouraging. Forty-five references are given.—C. E. D.

WILLHEIM, R., and F. BOCOBO. [Cancer Inst., Manila, Philippine Islands] EFFECTS OF HIGH DOSES OF NICOTINIC ACID ON HUMAN EPIDERMOID CARCINOMA. Acta Med. Philippina, 2:445-466. 1941.

Assuming that nicotinic acid, a constituent of the intracellular respiratory enzymatic system, may exert an influence on the metabolism of malignant cells, the authors administered 900 mgm. daily by mouth to patients with advanced squamous cell cancer of the oral cavity. In some instances 600 mgm. of methylene blue were added as an adjuvant. Biopsy before and after treatment disclosed considerable increase in keratinization of relatively undifferentiated tumors. Inflammatory reactions followed by a tendency to healing, and transitory local and general improvement accompanied the specific effect on the epithelial components of the tumors.—M. J. E.

RADIATION—DIAGNOSIS AND THERAPY

EDITORIAL. SERUM AND TISSUE PHOSPHATASE DETERMINATIONS IN RADIATION THERAPY. Am. J. Roentgenol., 45:757-759. 1941.

This editorial discusses the value of phosphatase determinations in following the progress of primary or metastatic malignant disease of bone. The work and opinions of Woodard and Higinbotham, (Am. J. Cancer, **31**:221-237. 1937. and J. A. M. A., **116**:1621-1627. 1941.) are cited extensively. A high serum phosphatase associated with an osteoplastic lesion or a normal serum phosphatase with an osteolytic lesion serves only to confirm the diagnosis. A normal serum phosphatase with an osteoplastic lesion suggests a slowly growing or relatively benign tumor. A high serum phosphatase associated with an osteolytic lesion suggests numerous possibilities among which are hyperparathyroidism and highly malignant osteogenic sarcoma. The trend of the serum phosphatase after radiation therapy of tumors involving bone shows a close correlation with the clinical course and indicates the degree and permanence of inactivation. Metastatic bone tumors affect serum phosphatase only indirectly in proportion to the degree of bony reaction to the invading tumor. Thus osteolytic metastases have little effect. Radiation therapy in metastatic lesions lowers an elevated serum phosphatase by suppressing the bony reaction.

When the tissue dose exceeds 4,000 r the phosphatase-producing mechanism of most bone tumors is inactivated. If an elevated serum phosphatase does not fall within 3 months after radiation therapy of a metastatic lesion, it indicates an inadequate dose or the development of new metastases.—C. E. D.

ERF, L. A., and J. H. LAWRENCE. [Univ. of California, Berkeley, Calif.] CLINICAL STUDIES WITH THE AID OF RADIOACTIVE PHOSPHORUS. I. THE ABSORPTION AND DISTRIBUTION OF RADIO-PHOSPHORUS IN THE BLOOD AND ITS EXCRETION BY NORMAL INDIVIDUALS AND PATIENTS WITH LEUKEMIA. J. Clin. Investigation, 20: 567-575. 1941.

The amount of radioactive phosphorus (P^{32}) retained by various fractions of the blood of 4 normal individuals, 12 patients with myeloid, and 15 with lymphoid leukemia, and the variations in retention following the administration of P^{32} when given orally and intravenously and when accompanied by varying amounts of nonradioactive phos-

phorus (P^{31}) have been determined. When P^{32} is administered orally, from 15 to 50% is excreted in the urine and feces in both normal individuals and patients during a 4 to 6 day period. In normal individuals the same percentages are excreted when P^{32} is administered intravenously, but in the patients only from 5 to 25% is excreted. When administered orally the greater part of the P^{32} is excreted in the feces, when intravenously a very small but definite amount is excreted in the feces. Normal individuals excrete large amounts in the urine following intravenous administration. In leukemic patients P^{32} is probably more quickly fixed in the pathological tissues and cells.

Marrow retains P^{32} in higher concentrations than blood per unit volume. Greater concentrations of P^{32} occur in the nuclei than in the cytoplasm of myeloid leukemic cells, while no differences in retention of P^{32} were noted in the nuclei and cytoplasm of the lymphoid cells studied. It is suggested that phosphorus passes from the acid-soluble substances of leukemic white blood cells, presumably through enzymatic carrier systems, to substances of nucleoprotein and phospholipid nature. If higher concentrations of P^{32} are to be obtained in circulating white blood cells, P^{32} should be introduced intravenously and it should be accompanied by the smallest amount of P^{31} possible.—J. L. M.

ERNST, E. C. [St. Louis, Mo.] THE RADIOLOGIC MANAGEMENT OF CANCER OF THE CERVIX. J. Missouri M. A., 38:267-271. 1941.

The author advocates preliminary roentgen therapy to reduce the inflammatory reactions invaribly accompanying cervical cancer, and as a supplement for subsequent treatment with radium. The effective quantity is measured by an ionization chamber inserted into the vagina, and sufficient radiation is administered through multiple ports to total 1,250 to 2,500 r within the tumor. Capsules of radium are inserted 1 to 3 weeks later in doses of 50 mgm. of the element intracervically and 25 mgm. laterally in each of the vaginal vaults. The radium is removed after 1 to 4 days.—M. J. E.

MURPHY, J. T., and C. E. HUFFORD. [Toledo, Ohio] CARCINOMA OF THE UTERINE CERVIX TREATED WITH 400 KV. ROENTGEN RAYS AND RADIUM. Am. J. Roentgenol., 45:801-803. 1941.

Sixty cases of carcinoma of the cervix followed for 3 to 5 years are presented. Survival at 3 years was 57% and at 5 years 30%. Administration of 1,800 to 2,850 r was given to anterior, posterior, and perineal portals preceded or followed by 4,000 mgm. hr. of radium. Damage to the bowel rather than skin damage was the limiting factor in roentgen ray dosage. Acute roentgen sickness was not an important factor but some patients showed an unexplained reaction characterized by loss of appetite and strength, and general breakdown. Vaginal adhesions and constriction and pelvic fibrosis were prone to occur, rendering pelvic examination for recurrence difficult. Bladder symptoms were few and generally mild but several vesicovaginal fistulas were encountered. Proctitis and pelvic colitis occurring 3 to 6 months after treatment was an annoying but not a serious complication.—C. E. D.

PEPLE, W. L. [Richmond, Va.] THE RESULTS OF RADIUM TREATMENT OF CANCER OF THE CERVIX. South. Surgeon, 10:600-606. 1941.

In a series of 164 patients with cancer of the cervix treated with radium, of which 121 had adequate follow-up records or did not die of intercurrent disease, 43(36%) were classified as cured 5 or more years after the conclusion of therapy.—M. J. E.

Skin and Subcutaneous Tissues

ANDRES, G. G., and E. I. AVDUSINA. [Central Oncological Inst., Moscow] CONTRIBUTIONS TO THE CARYOTYPE OF THE SOMA OF ADULT MAN. V. ON THE CARYOLOGY OF CERTAIN PRECANCEROUS STATES OF THE SKIN. Bull. biol. et méd. expér. URSS., 5-6:504-506. 1940.

The material studied was obtained from the following cases (1) ulcer of the lower lip, (2) hyperkeratosis of the lower lip, (3) hyperkeratosis of the skin. The tissues were fixed in Flemming, embedded in paraffin, and stained after Newton. The ratios of the normal mitotic phases varied to some extent among the 3 cases, but were probably due to chance fixation at various stages of mitosis. The proportion of aberrant mitoses was insignificant (0.8% to 1.8%). In 19 nuclei a chromosome count was made. Eighteen nuclei had 46 to 50 chromosomes; one nucleus showed 56 chromosomes. Hyperploidy in the latter case was mainly associated with premature chromosomal splitting and disjunction. Thus if hyperkeratosis is considered a precancerous condition, it does not produce quantitative chromosome variations characteristic of malignant growth. The appearance of individual hyperploid cells, however, may signify a further stage of a progressing malignant condition.—M. B.

LOEB, M. J. [Bronx Hosp., Bronx, N.Y.] TRAUMA AND GLOMUS TUMORS: RELATIONSHIP, WITH REPORT OF A CASE. Indust. Med., 10:208-213. 1941.

Pain in the subungual region of a ring finger, apparent soon after an injury, persisted for 8 years and was relieved by excision of a glomus tumor.—M. J. E.

PRATES, M. [Inst. Portugués de oncologia, Lisbon] SOBRE TUMORES SUPERFICIAIS INTRA-EPITELIAIS. [CONCERNING SUPERFICIAL INTRA-EPITHELIAL TUMORS.] Arq. de pat., 12:545-565. 1940.

Theoretically a skin epithelioma could propagate through the epidermis and destroy it without breaking through the basal membrane, although rarely has any such case been observed. Borst, in 1904, described the first case and together with those reported by Jadasshon and Montgomery 5 cases of such tumors are known. The author reports 4 more cases, 2 of them observed in Berlin's Charité and the other 2 at the Portuguese Institute. The tumors were located in the face, nipple, and thigh, were very radioresistant, and were treated surgically. Complete clinical histories with histological studies illustrated by 16 photographs are appended.—M. D-R.

SEWELL, R. L. [Univ. of Roch. Sch. of Med. and Dentistry, Rochester, N. Y.] BASAL CELL CARCINOMA IN YOUTH. Arch. Surg., 42:909-912. 1941.

A basal cell carcinoma occurring in the scalp of a girl of 13 years is described. The prognosis seems as favorable as in the older age groups.—G. De B.

Nervous System

BROWN, M. H., and J. W. KERNOHAN [Mayo Clinic, Rochester, Minn.] DIFFUSE MENINGIOMATOSIS. Arch. Path. 32:651-658. 1941.

A case of diffuse meningiomatosis is reported and its clinical and pathological implications are discussed. The process resembles a neoplastic transformation of multicentric origin arising in an incompletely differentiated arachnoid membrane.—Authors' summary.

JAMES, A. G., and G. M. CURTIS. [Ohio State Univ., Columbus, Ohio] MEDIASTINAL GANGLIONEUROMA. Ann. Surg., 113:767-777. 1941.

The author adds a new case to the 33 cases of this rare tumor reported in the literature. The patient, a 35-year-old female with a posterior mediastinal tumor filling the right lower chest, was alive and well 20 months after successful surgery. These tumors are of sympathetic nervous system origin. They are well differentiated and of low-grade malignancy. A classification of tumors of the sympathetic nervous system is quoted.—A. M.

KLEMME, R. H., and R. D. WOOLSEY. [St. Louis Univ., St. Louis, Mo.] TUMORS OF THE BRAIN: CASE REPORT. J. Missouri M.A., 38:282-284. 1941.

An extracortical tumor of the right parietal region of a 20-year-old youth recurred 7 months after excision. It was considerably larger than the primary growth and infiltrated the temporal muscle. A second recurrence developed after partial removal, and when the patient died, the tumor had invaded the bony structures, muscle, and skin of the side of the face. Histologically it appeared to be a sarcoma, possibly of meningeal origin.—M. J. E.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27201. New England J. Med., 224:863-866. 1941.

This is a case report of a 62-year-old male operated upon for a brain tumor. The differential diagnosis is discussed. A diagnosis was given of "hemangioma" of the posteroparietal region of the left hemisphere. However, post-mortem examination revealed direct arterial communications with the superior longitudinal sinus which suggested a developmental anomaly rather than a true neoplasm.—A. M.

MERWARTH, H. R., and E. FEIRING. [Brooklyn Hosp., Brooklyn, N. Y.] TUMOR OF THE BRAIN SIMULATING ENCEPHALITIS. Brooklyn Hosp. J., 3:221-232. 1941.

Clinical report. Because of an early fever and an increased cellular count in the cerebrospinal fluid, a case of primary tumor of the pons was found to simulate encephalitis.—J. L. M.

RAAF, J. [Portland, Oreg.] A PLEA FOR THE EARLY DIAGNOSIS OF SPINAL CORD TUMORS. West. J. Surg., 49:147-151. 1941.

A report of 4 cases, 2 of benign tumor successfully extirpated (meningioma, perineural fibroblastoma), and 1 each of metastatic myeloma and inoperable ependymoma, illustrates the need for earlier diagnosis in patients with vague clinical evidence of neoplastic disease involving the spinal cord.—M. J. E.

YAMADA, C. [Aus der Frauenklinik der Kaiserlichen Tohoku-Univ., Sendai, Japan] ÜBER EINEN FALL VON RETROPERITONEALEM GANGLIONEUROM. [A CASE OF A RETROPERITONEAL GANGLIONEUROMA.] Gann, 35: 148-151. 1941.

The detailed clinical and necropsy findings of a case of a retroperitoneal ganglioneuroma in a 14-year-old girl are reported. According to the author, this represents the 54th case to be reported in the literature. The cases are classified as to age and sex of patient, site of tumor, etc., and are discussed.—P. P. C.

Ear

DANDY, W. E. [Johns Hopkins Univ. Sch. of Med., Baltimore, Md.] RESULTS OF REMOVAL OF ACOUSTIC TUMORS BY THE UNILATERAL APPROACH. Arch. Surg., 42:1026-1033. 1941.

Since the introduction of the unilateral approach to these tumors, the author has totally removed 46 such neoplasms with 5 deaths, a mortality of 10.87%, but there has been no death in the last 16 cases. Except for 2 deaths, the rest are probably living and well. There has been no recurrence of the lesion. Following the operation, there is usually paralysis of the facial nerve, rare and temporary injury to the vagus nerve, and always loss of the acoustic nerve. The size of the tumor is an important index of the risk assumed in the operation.—G. De B.

Breast

GESCHICKTER, C. F. [Baltimore, Md.] THE ENDOCRINE ASPECTS OF CHRONIC CYSTIC MASTITIS. South. Surgeon, 10:457-486. 1941.

The clinical entity, chronic cystic mastitis or mammary dysplasia, consists of 3 distinct pathological processes: mastodynia, characterized by defective lobule formation disorganized by proliferating connective tissue; adenosis, a more advanced stage, in which small cysts are associated with intraductal and intracystic epithelial proliferation; cystic disease, dominated by the formation of large isolated, fibrotic, blue-domed cysts accompanied by lobular involution. The first types occur in the thirties or early forties especially in women who are childless or have not borne children recently, the third occurs during the menopause. If pregnancy supervenes, a favorable influence on the mammary alterations is commonly observed. This observation, in conjunction with the finding that reduced quantities of pregnandiol were demonstrable in 48-hour urine specimens of patients with mammary dysplasia, while the estrogen excretion was normal, leads to the supposition that the mammary changes depend upon an absolute or relative decrease in the elaboration of the corpus luteum hormone associated with hyperestrinism. Treatment with progesterone exerted a favorable effect, except in the type with large cysts. Spontaneous remissions are not uncommon, while testosterone also may alleviate symptoms. Estrogens frequently produce symptomatic and objective amelioration, possibly through stimulation of luteal function. The hormonal relationships of normal and pathological mammary development to ovarian secretion are, therefore, still uncertain. Excessive estrogen does not invariably produce cancer in abnormally proliferating breast tissue.

Opinion differs widely as to whether chronic cystic mastitis is a precursor of mammary cancer. The author's

observations tend to negate a close relationship of the two conditions. Mammary dysplasia is not essential to the appearance of cancer and, when it occurs, it does not necessarily assume the role of predisposing factor to malignant alteration. While microscopic evidence of chronic cystic mastitis is common in cancerous breasts, similar changes may be found in from 25 to 93%, depending upon the age, of subjects with apparently normal breast tissue obtained routinely at necropsy. On the basis of studies of vital statistics the author reports that among 2,500 personally observed cases of mammary cancer and 1,200 of chronic cystic mastitis, instead of a calculated coexistence of the two lesions in 27 cases, the observed number was 64. Slightly higher percentages in the incidence of cancer were likewise recorded in groups of women observed for periods of 5 to 15 years following the diagnosis of chronic cystic disease than in the general female population.

Pathologic states simulating mammary dysplasia may be produced in castrated female rats by prolonged administration of estrogen, and progesterone or testosterone, but with an excess of the first hormone. Mammary cancer arises in animals so treated only if, in addition, exceptionally large doses of estrogen are given during senility. The female sex hormone alone, when injected at an early age, is more likely to give rise to distortion of the mammary ducts, the formation of large cysts, and in some instances early cancer.—M. J. E.

LEIGHTON, W. E. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] BREAST CANCER: A NEW INCISION FOR RADICAL OPERATION. J. Missouri M.A., 38:274-275. 1941.

Description of surgical procedure.—M. J. E.

LEUCUTIA, T. Editorial. IRRADIATION AND THE ESTROGENIC THEORY OF MAMMARY CARCINOMA. Am. J. Roetgenol., 45:923-925. 1941.

Arguments are advanced favoring the theory that estrogenic stimulation accounts for the development of breast cancer. Thorough local irradiation is advised in all cases whether or not surgery is feasible. The value of castration is still undetermineed.—C. E. D.

Female Genital Tract

ARNESON, A. N. [Washington Univ. Sch. of Med., St. Louis, Mo.] CANCER OF THE CERVIX UTERI: BIOLOGICAL FACTORS IN TREATMENT. J. Missouri M.A., 38:272-273. 1941.

Two chief forms of cancer of the cervix are distinguished: a vascular, friable, cauliflower type, and a relatively avascular, infiltrating, more desmoplastic variety. The former in general is radiosensitive, the latter more resistant. In evaluating the action of radiation it is important to distinguish the effects on the cancer cells and the tumor bed. Excessive treatment may produce considerable necrosis, fibrous tissue repair, and eventually a resistant tumor. As infection lowers the threshold for necrosis, it is imperative to combat this complication with judiciously administered roentgen radiation, cleansing irrigations, and antiseptic solutions.—M. J. E.

BOWING, H. H., and J. A. L. McCULLOUGH. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE CERVIX UTERI IN CHILDHOOD AND ADOLESCENCE: A REVIEW OF THE LITERATURE AND REPORT OF AN ADDITIONAL INSTANCE OF THE LESION IN A GIRL AGED THIRTEEN. Am. J. Roentgenol., 45:819-826. 1941.

A case of adenocarcinoma of the cervix occurring in a 13-year-old girl is reported. A six year cure without destroying menstrual function resulted when 676 mc.hr. of radon was applied to the cervical canal. A review of the literature since 1862 revealed only 25 instances of cervical carcinoma in girls under 20. These reports are briefly summarized and tabulated. Only 12 are considered proved.

This and the three preceding papers on cancer of the cervix are discussed together by Drs. M. Lenz, W. P. Healy, A. N. Arneson, H. J. Ullmann, D. Y. Kieth, H. E. Schmitz, J. T. Murphy, H. G. F. Edwards, and F. J. Taussig.—C. E. D.

EDWARDS, H. G. F. [Tumor Clinic, Shreveport Charity Hosp., Shreveport, La.] CARCINOMA OF THE CERVIX UTERI: A STUDY OF 727 CASES. Am. J. Roentgenol., 45: 804-812. 1941.

Nine hundred and five cases of cancer of the female genital tract are presented of which 727 cases were of the cervix. Of these, 265 patients were white and 462 colored. The cervical cancers were treated by roentgen irradiation in doses of 1,200 to 2,400 r delivered to each of 4 portals supplemented in about half the cases by radium. Many of the patients who came to autopsy showed local cure of the disease and died from extrapelvic metastases. Twenty per cent of the patients survived 5 or more years, even though over 75% were first seen in an advanced stage of disease. A plea is made for earlier diagnosis and treatment and a semiannual pelvic examination is suggested for all women over 35.—C. E. D.

HAGER, B. H., and E. O. BOETTICHER. [Los Angeles, Calif.] A DEGENERATING MALIGNANT MYOMA OF THE UTERUS WITH VESICAL FISTULA PREVIOUSLY CONSIDERED MEMBRANOUS INFLAMMATION OF A URETERAL STUMP. J. Urol., 46:542-544. 1941.

Case report.—H. G. W.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27172. New England J. Med., 224:745-747. 1941.

A women 52 years old received x-ray therapy for carcinoma which developed in a cervical stump 12 years after hysterectomy for fibromyomas. Six months later she succumbed to uremia. Autopsy revealed extensive involvement of the bladder, occlusion of both ureters, metastases to kidney and lungs, and a vesico-vaginal fistula. —A. M.

SCHATTENBERG, H. J., and J. ZISKIND. [Tulane Univ. Sch. of Med., New Orleans, La.] EARLY CARCINOMA OF THE CERVIX. Am. J. Clin. Path., 11:719-729. 1941.

A discussion of the recognition of pre-invasive carcinoma of the cervix, with changes in the epithelium characteristic of carcinoma cells but no invasion through the basement membrane. These lesions are considered to be true carcinomas, but amenable to less radical treatment than is needed for carcinoma with invasion.—H. G. W.

SMITH, G. VAN S. [Harvard Med. Sch., Boston, Mass.] CARCINOMA OF THE ENDOMETRIUM. A REVIEW WITH RESULTS THROUGH 1935. New England J. Med., 225:608-615. 1941.

The author reviews 307 cases of proven primary endometrial carcinoma, first treated at The Free Hospital for Women in Brookline.

Eighty-five per cent were postmenopausal; 51% were nulligravida. Three had had ovariectomy from 15 to 30 years before admission. Many in the series were mismanaged (9%), ergot, ovarian surgery, and x-ray therapy being employed for vaginal bleeding, without benefit of biopsy.

Three tables summarize results from 1903-25, 1926-30, and 1931-35 respectively. The total 5-year salvage figures are given as 54.8%, 56.0%, and 56.2%, indicating little improvement in results. The contribution of radiation therapy, employed since 1930, seems uncertain. Complete hysterectomy was performed in 77 of 112 patients treated (1931-35). In 15, receiving no therapy other than surgery, 9 (60%) were well after 5 years. These percentages in the other two groups (1903-25, 1926-30) were 63 and 67. The remaining 62 cases received some form of radiation therapy and 63% were well after 5 years. There were 11 who received only postoperative x-ray with 9 (82%) 5-year survivals. Of interest is the group of 17 treated by the sequence of radium, hysterectomy, x-radiation; only 53% survived 5 years. Yet the latter therapy is widely accepted today.

Five per cent of the 307 patients had other malignant diseases, including 5 with breast and 8 with ovarian carcinoma. More than 10% had benign ovarian tumors including 5 in the granulosa-theca cell series. Evidence of increased stromal or thecal cell activity occurred in 87% of postmenopausal ovaries, suggesting the possible etiologic role of continued estrogenic stimulation.—A. M.

TAUSSIG, F. J. [St. Louis, Mo.] RESULTS IN TREATMENT OF LYMPH NODE METASTASIS IN CANCER OF THE CERVIX AND THE VULVA. Am. J. Roentgenol., 45: 813-818. 1941.

Lymph node metastases of cervical or vulvar carcinoma are rarely cured by radiation. Surgical removal of regional nodes gives fairly good results. In cancer of the cervix, the presence of metastases reduces the number of cures to about ½ that of the cases without metastases. If a complete Basset operation is done in cancer of the vulva, the presence of lymph node metastases reduces the percentage of cures from 63.6 to 52.6%. Three case histories are given illustrating long survival after treatment of lymph node metastases.—C. E. D.

WILSON, J. M. [Duke Hosp., Durham, N. C.] BASAL CELL CARCINOMA OF THE VULVA. Arch. Surg., 43:101-112. 1941.

Four cases of primary basal-cell carcinoma of the vulva are reported, together with 23 cases collected from the literature. The general incidence varies considerably, being 12% of 32 cases of primary carcinoma of the vulva at the Duke Hospital. The death rate from the tumor itself is estimated to be at least 36% in the entire series, but it is believed that the prognosis is better than the squamous cell type of carcinoma. When possible, wide local excision should be done. The tumor metastasizes infrequently.—G. De B.

ZISKIND, J., and H. J. SCHATTENBERG. [Tulane Univ. Sch. of Med., New Orleans, La.] SARCOMA OF THE UTERUS. New Orleans M. & S. J., 93:637-641. 1941.

Case report of an endometrial sarcoma, which is less common and more malignant than the muscle tissue tumors.—H. G. W.

Ovary

CURTIS, A. H. [Northwestern Univ. Med. School, Chicago, Ill.] THECA CELL TUMORS (THECOMA). Surg. Gynec. & Obst., 73:481-483. 1941.

The 34th recorded case of thecoma is presented. The fundamental characteristic is a connective tissue derivation with a high lipoid content in an ovarian tumor. Malignancy is rare, and they occur chiefly in women beyond the menopause with atypical bleeding. There is a moderate amount of lipoid material chemically with a high percentage of cholesterol and a large amount of estrogen.—H. G. W.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27161. New England J. Med., 224:694-698. 1941.

A woman of 53 entered with a bloody vaginal discharge of 6 months' duration. She had hirsutism, hypertension, an enlarged clitoris, and a diabetic sugar tolerance curve. Examination under anesthesia showed normal adnexae and endometrium. Six months later she was readmitted with vaginal bleeding and generalized acne. An adenoma testiculari of the right ovary, 3 cm. in diameter, was found.—A. M.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27411. New England J. Med., 225:585-588. 1941.

A case of papillary cystadenocarcinoma of the ovary with extensive calcification of peritoneal and pleural metastases. X-ray evidence of calcification, which was due to "psammoma body" formation, was present for 6 years.—A. M.

Male Genital Tract

GILBERT, J. B. [Schenectady, N. Y.] STUDIES IN MALIGNANT TESTIS TUMORS. V. TUMORS DEVELOPING AFTER ORCHIDOPEXY. J. Urol., 46:740-747. 1941.

A report of 2 cases and a review of 63 cases of tumors developing after orchidopexy, of which 19 were teratoid tumors and 34 were unicellular. The survival rate for this group is poor.—H. G. W.

KATZEN, B. [Jewish Hosp., Brooklyn, N. Y.] METASTATIC CARCINOMA OF THE EPIDIDYMIS. J. Urol., 46: 734-739. 1941.

Only 2 cases have been reported previously. An additional case is recorded in which the primary tumor was an adenocarcinoma of the stomach.—H. G. W.

Urinary System—Male and Female

CLANCY, F. J. [Seattle, Wash.] NEOPLASM COMPLICATING DIVERTICULUM OF THE BLADDER. J. Urol., 46:486-490. 1941.

A case is added to the 50 recorded in the literature.—H. G. W.

COLLINGS, C. W., and F. WELEBIR. [Coll. of Med. Evangelists, Los Angeles, Calif.] J. Urol., 46:494-497. 1941. OSTEOMA OF THE BLADDER.

A unique case of osteoma of the urinary bladder is reported.—H. G. W.

GREENFIELD, H. [New York, N. Y.] DISTANT METASTASES FROM CARCINOMA OF THE URINARY BLADDER. Radiology, 37:181-185. 1941.

Metastases from carcinoma of the urinary bladder occur late, if at all, and are often confined to the pelvis. The

size of the local tumor is no indication of the likelihood of metastasis. Four of the author's 68 cases had distant metastases demonstrable during life. Case reports are given without autopsies.—C. E. D.

KESSLER, E. E. [Los Angeles, Calif.] PAPILLARY CYSTADENOMA OF THE KIDNEY. J. Urol., 46:505-509. 1941.

A case report.—H. G. W.

ROBERTSON, T. D., and J. R. HAND. [Portland Clinic, Portland, Oreg.] PRIMARY INTRARENAL LIPOMA OF SURGICAL SIGNIFICANCE. J. Urol., 46:458-474. 1941.

Twelve cases of primary intrarenal lipoma, removed surgically, have been collected from the literature and two additional cases are reported. They may be clinically indistinguishable from carcinoma, although of good prognosis. They may contain connective tissue, smooth muscle, myxomatous vascular tissue, and cartilage. It is suggested that they arise from embryonic connective tissue surrounding the developing collecting tubules.—H. G. W.

SEAMAN, J. A., and C. BINNIG. [Westfield State Hosp., Springfield, Mass.] UROLOGICAL COMPLICATIONS OF CANCER OF THE RECTUM. J. Urol., 46:777-787. 1941.

An analysis of bladder conditions met with after operations for cancer of the rectum in 68 patients.—H. G. W.

ZUCKER, M. O., and G. J. WEINSTEIN. [Lincoln Hosp., New York, N. Y.] PRIMARY CARCINOMA OF THE URETHRA IN THE MALE. REPORT OF A CASE. New England J. Med., 225:682-684. 1941.

A 59-year-old male, with an old history of gonorrheal urethritis, was treated for an intractable stricture for 5 years. Carcinoma was not suspected until perineal fistulae appeared. He succumbed 1 month after the diagnosis was made.—A. M.

Gastrointestinal Tract

LAWRENCE, K. B. [Pondville Hosp. at Norfolk, Wrentham, Mass.] BASAL CELL EPITHELIOMA OF ANUS. Arch. Surg., 43:88-93. 1941.

Two cases of basal cell epithelioma are added to the literature, which previously contained only 4 cases. For bulky tumors, treatment should consist of posterior resection of the anorectum, with or without postoperative irradiation. For the smaller tumors, radium therapy and irradiation are sufficient.—G. De B.

Liver

BRUNSCHWIG, A., AND D. E. CLARK. [Univ. of Chicago, Chicago, Ill.] CARCINOMA OF CYSTIC DUCT. Arch. Surg., 42:1094-1100. 1941.

Removal of this carcinoma of the cystic duct entailed resection of a segment of the common duct and of the main hepatic artery. Death followed in 22 days after operation and was attributed to failure of the liver, incident to interruption of the hepatic artery.—G. De B.

WALLACE, R. H. [Pondville Hosp. at Norfolk, Wrentham, Mass.] RESECTION OF THE LIVER FOR HEPATOMA. Arch. Surg., 43:14-20. 1941.

A patient with a large solitary liver cell hepatoma is reported alive and well more than 5 years following partial resection of the liver.—G. De B.

Muscle and Tendon

AITKEN, A. P. [Boston, Mass.] ROENTGENOGRAPHIC RECOGNITION OF SYNOVIOMA. J. Bone & Joint Surg., 23:950-952.

A case report confirming the statement of R. W. Lewis that a soft-tissue tumor shadow near a joint containing a scattered deposit of amorphous lime, is characteristic of synovioma.—H. G. W.

Oral Cavity and Upper Respiratory Tract

BRIGHTON, G. R., F. ALTMANN, and C. HAGAN, JR. [Presbyterian Hosp., New York, N. Y.] REACTIONS OF LARYNGEAL TISSUES FOLLOWING EXTENDED FRACTIONAL ROENTGEN IRRADIATION. Arch. Otolaryng., 33: 631-658. 1941.

The larynxes of 16 patients who received protracted fractional roentgen therapy for laryngeal cancer were examined for the early and late reactions to radiation of normal and neoplastic tissues. The total surface dose varied from 2,400 to 9,000 r administered generally through bilateral cervical fields. Reactions observed between 4 and 51 days after the termination of treatment were considered as representative of early stages, while those occurring after 3½ months to 2½ years were grouped as late manifestations. The most prominent early alterations were necrosis of the superficial epithelium of the larynx, degeneration of the glandular tissue, pseudomembranous inflammatory reactions, and endothelial proliferation and thrombosis in the small blood vessels. Healing occurs in later stages and is characterized by epithelial regeneration often accompanied by a transformation of the originally ciliated epithelium to a squamous type with zones of excessive cornification, obliteration of the affected blood vessels, atrophy of the glandular elements, and fibrosis. Cartilage, bone, and muscle are generally unaffected. Degeneration of the neoplastic cells and subsequent fibrous tissue replacement were the prominent features in the irradiated tumors.—M. J. E.

JACKSON, C. L. [Temple Univ. Sch. of Med., Philadelphia, Pa.] LARYNGOFISSURE FOR CANCER OF THE LARYNX: OBSERVATIONS BASED ON A SERIES OF FIFTY CONSECUTIVE CASES. Arch. Otolaryng., 33:520-535. 1941.

Laryngofissure is a suitable operation in selected cases of intrinsic cancer of the larynx. The operative mortality is negligible and the functional result satisfactory. There were no immediate fatalities in the present series, but 6 patients died 1 to 3 years after operation of causes other than cancer. A recurrence, necessitating a second surgical intervention, developed in only 5 patients, but 1 of this group died of postoperative complications. Illustrations of surgical methods are included.—M. J. E.

JORSTAD, L. H. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] TREATMENT OF LINGUAL CANCER. J. Missouri M.A., 38:279-281. 1941.

As partial or complete glossectomy was associated with a high operative mortality and rarely effected a permanent cure, surgery has been supplanted largely by radiation in the treatment of cancer of the tongue. Surgical measures, however, were employed in 20 cases, and only 3 patients survived tumor-free after 5 years. On the other hand 39 of 64 patients in whom radon seeds were implanted remained cured for this minimum period. In the remaining 24 cases roentgen therapy or radium was

employed without success. In all groups dissection of the neck was combined with local treatment, only if metastatic foci were present. The importance of early diagnosis is demonstrated by the fact that 65% of the patients cured with radon had no metastases at the time of treatment.—M. J. E.

KEMLER, J. I. [Baltimore, Md.] NITROGEN MONOXIDE AND OXYGEN FOR ANAESTHETIZATION IN OPERATIONS FOR MALIGNANT GROWTHS OF THE LARYNX. Arch. Otolaryng., 33:707-710. 1941.

Description of method.—M. J. E.

NEW, G. B., and O. E. HALLBERG. [Mayo Clinic, Rochester, Minn.] THE END RESULTS OF THE TREATMENT OF MALIGNANT TUMORS OF THE PALATE. Surg., Gynec. & Obst., 73:520-524. 1941.

A review of 173 cases of malignant tumors observed in the Mayo clinic between 1907 and 1939; at the same time 236 benign tumors were observed. Of the malignant tumors, 117 were in males and 56 in females. Squamous cell carcinoma accounted for 84 cases, adenocarcinoma for 76, and 13 were miscellaneous types. Of the adenocarcinoma patients who were traced, 88% lived 5 years, and 78% lived over 10 years, whereas only 20% of the traced patients with squamous-cell carcinoma lived over 5 years.—H. G. W.

TUCKER, G. [Philadelphia, Pa.] CANCER OF THE LARYNX, DIAGNOSIS, TREATMENT AND RESULTS: WITH OBSERVATIONS ON THE RELATION OF BENIGN TUMORS TO CANCER. South. Surgeon, 10:671-679. 1941.

General clinical remarks.—M. J. E.

Intrathoracic Tumors—Lungs—Pleura

BETTS, R. H. [New England Deaconess Hosp., Brookline, Mass.] CARCINOMA OF THE LUNG: BRONCHOSCOPIC ASPECTS. New England J. Med., 225:519-525. 1941.

The author emphasizes the valuable role of the bronchoscope in the diagnosis of bronchial carcinoma. In a personal series of 62 cases the author was able to visualize and biopsy 74%. Bronchoscopy ought not now to be regarded as an ordeal. Its use is indicated for all patients with unexplained pulmonary symptoms. Where x-ray suggests tumor, it provides more accurate details as to type and operability. The instrument's therapeutic possibilities are also mentioned. The article includes 3 x-rays, 3 diagrams, and a bibliography of 14 papers.—A. M.

BLADES, B. [Washington Univ. Sch. of Med., St. Louis, Mo.] RELATIVE FREQUENCY AND SITE OF PREDELICTION OF INTRATHORACIC TUMORS. Am. J. Surg., 54: 139-148. 1941.

General considerations, with particular reference to the tumors seen at the Barnes Hospital.—H. G. W.

BRUNN, H., and A. GOLDMAN. [Univ. of Calif. Med. Sch., San Francisco, Calif.] BRONCHIAL ADENOMA. Am. J. Surg., 54:179-192. 1941.

Based on 19 cases personally observed and 40 cases personally reviewed in various clinics, a study of the various types of bronchial adenomas is presented. In striking contrast to carcinoma of the bronchus is the high incidence of bronchial adenoma in women, 63% of the authors' cases having been in women. An earlier age incidence also obtains. The authors' conception of their pathogenesis is that bronchial adenomas arise from the mucous glands and their ducts, which, being mixed glands, are apt to have varying patterns, and in general they follow the behavior of adenomatous tumors arising from these glands elsewhere in the body. Bone and cartilage formation is probably the result of de-differentiation of adult tumor cells. Three morphological types occur: endobronchial, intramural, and extrabronchial, of which the intramural and extrabronchial constitute at least 90%—H. G. W.

COTTON, B. H. [New England Deaconess Hosp., Boston, Mass.] DIFFERENTIAL DIAGNOSIS OF PRIMARY AND METASTATIC MALIGNANCY OF THE LUNG. Am. J. Surg., 54:173-178. 1941.

Clinical study.—H. G. W.

GARLOCK, J. H. [Mount Sinai Hosp., New York, N. Y.] THE PRESENT STATUS OF THE SURGICAL TREATMENT OF CARCINOMA OF THE THORACIC ESOPHAGUS. Am. J. Surg., 54:262-272. 1941.

Of 30 squamous cell carcinomas of the esophagus, 19 were considered operable and were resected with an operative mortality of 8, and 6 survivors from 5 months to 5 years. Of adenocarcinomas of the cardia with esophageal involvement there were 24 cases, of which 9 were found operable and resected, with 4 operative deaths, and 4 survivors from 5 to 16 months.—H. G. W.

HALPERT, B. [Louisiana State Univ. Med. Sch., New Orleans, La.] CARCINOMA OF THE LUNG. A TEN YEAR SURVEY OF NECROPSIES IN THE CHARITY HOSPITAL AT NEW ORLEANS. J.A.M.A., 117:1090. 1941.

During the past 10 years carcinoma of the lung was discovered in 135 of 8,862 necropsies in persons over 1 year of age, increasing almost steadily from 3 in the first year to 30 in the last. It ranks second to carcinoma of the stomach, which accounted for 205 deaths, and in the last 2 years was exceeded by pulmonary cancer. It was about 10 times as frequent in men as in women, and the ratio in white exceeded the ratio in colored patients by about 3 to 2. More than 50% were squamous cell, 30% were reserve cell, and less than 20% columnar cell.—H. G. W.

JANES, R. M. [Toronto, Canada] TUMORS OF THE THORACIC CAGE. Am. J. Surg., 54:127-138. 1941.

Tumors of the chest wall comprise all growths affecting tissues of similar character elsewhere in the body. While they do not present any special pathological characteristics they do, because of their location, present special surgical problems. Because they are infrequent, case reports of 15 cases of primary lesions of the ribs and cartilages are given in detail.—H. G. W.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27191. New England J. Med., 224:822-824. 1941.

A case report of a 61-year-old male with dyspnea and fatigability for 2 years and hemoptysis for 8 months. X-ray revealed a large mass with a calcified margin occupying the anterior aspect of the left chest. A mediastinal dermoid cyst was successfully removed.—A. M.

MALLORY, T. B., Editor [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27192. New England J. Med., 224:824-827. 1941.

A case of highly undifferentiated bronchiogenic carcinoma, "probably squamous cell in type," with mediastinal and cerebral metastases, in a 45-year-old male.—A. M.

OVERHOLT, R. H. [New England Deaconess Hosp., Boston, Mass.] CARCINOMA OF THE LUNG AS A SURGICAL PROBLEM. Am. J. Surg., 54:161-172. 1941.

Carcinoma of the lung has become of vital concern to every practicing physician because it is near the top of the list of cancer deaths, it is curable in its early stages, because methods are available for detecting early cases, and there is reason to believe that the majority of patients consult a doctor at a time when the lesion is in an operable stage. Of 174 patients suspected of having cancer of the lung, tissue verification was obtained in 127, of which 47 were apparently operable, and of which 13 are now living with no evidence of metastases.—H. G. W.

Bone and Bone Marrow

CAMPBELL, W. C., and J. F. HAMILTON. [Memphis, Tenn.] GRADATION OF EWING'S TUMOR (ENDOTHELIAL MYELOMA). J. Bone & Joint Surg., 23:869-876. 1941.

Since there is no recorded attempt to grade Ewing's tumor, an attempt was made to grade 30 cases. The nuclear chromatin content and the cell activity, as measured by the number of mitotic figures per high power field, seemed to be the most significant finding. Tumors with cells containing a large number of chromatin knots are probably more malignant than those containing a smaller number.—H. G. W.

GHORMLEY, R. K., and A. W. ADSON. [Mayo Clinic, Rochester, Minn.] HEMANGIOMA OF VERTEBRAE. J. Bone & Joint Surg., 23:887-895. 1941.

A study of 39 patients, of whom 27 or 69% were females. Although often hemangioma of the vertebrae does not cause symptoms, sometimes severe symptoms are present, even complete paraplegia which requires laminectomy followed by roentgenotherapy.—H. G. W.

PADGETT, E. C., and N. B. SODERBERG. [Univ. of Kansas Sch. of Med., Kansas City, Mo.] ADAMANTINOMA OF THE JAW. J. Missouri M.A., 38:276-279. 1941.

Tumors varying in diameter from 1 to 10 cm. were extirpated successfully from the mandible of 5 patients and the superior maxilla of 2. Radiotherapy had been attempted previously in 3 cases without apparent benefit.—M. J. E.

Leukemia, Lymphosarcoma, Hodgkin's Disease

ARONS, I. [New York, N. Y.] FURTHER STUDIES ON RADIOTHERAPY OF LYMPHOBLASTOMA. Radiology, 37: 164-173. 1941.

The term lymphoblastoma is used to include lymphosarcoma, lymphadenoma, Hodgkin's disease, and lymphatic leukemia. Literature is cited to show the close relationships among these conditions and the general usefulness of radiation therapy. Eight case histories are presented from a group of 45 patients treated by the author. Treatment should be extensive enough to reach all demonstrable lesions as well as those regions known to be frequently infiltrated. Prophylactic irradiation is discouraged in the absence of active foci of disease. General irradiation should be used only in selected cases as a supplement to local treatment. An argument is advanced that normal activity of the reticulo-endothelial system is a major factor in resistance against malignant growth. Hence radiation damage to this system should be held to a minimum by using the smallest doses capable of destroying the malignant cells. Proper treatment may treble life expectancy in lymphoblastoma.—C. E. D.

BUDD, J. W. [Los Angeles, Calif.] NEOPLASMS OF THE RETICULOENDOTHELIAL TISSUES. California & West. Med., 55:84-86. 1941.

The variations in the clinical and pathological aspects and the success of therapy of reticulum cell sarcoma are illustrated by a report of 6 cases. Diagnosis in each was ascertained by biopsy, operation, or necropsy. The spleen was involved primarily in 2 cases, the skeletal system in 3, and the stomach in 1. The splenic reticulocytoma was extirpated in both instances. One patient died subsequently of monocytic leukemia, the second had a metastasis to the spinal dura, but symptomatic relief was achieved with roentgen therapy. The osseous type with single or multiple foci also responded to radiation, but of the 2 patients benefited 1 died suddenly of neoplastic erosion of the splenic artery. Finally an apparently inoperable gastric cancer proved to be a reticulum cell sarcoma and roentgen therapy caused complete regression of the mass.—M. J. E.

ERF, L. A. [Crocker Radiation Lab. Univ. of California, Berkeley, Calif.] RETENTION OF RADIOPHOSPHORUS IN WHOLE AND ALIQUOT PORTIONS OF TISSUES OF PATIENT DEAD OF LEUKEMIA. Proc. Soc. Exper. Biol. & Med., 47:287-289. 1941.

Nineteen days before death, the patient took orally 120 cc. of a solution containing sodium phosphate (radioactive phosphorus) and emitting 20 mc. of beta radiation. At post-mortem examination, 38 organs and tissues were weighed, as were small aliquots (about 5 gm.) of each. The whole organs, tissues, and their aliquots were placed in separate crucibles and ashed at 400° C. The ashes of the aliquots were immediately measured for radioactivity by means of an electrometer. The ashes of the whole organs and tissues were thoroughly mixed and 100 mgm. aliquots assayed for radioactivity.

The variations found in the P^{32} content of the ash of the whole organs and their respective aliquots is probably due to the fact that small aliquots of organs cannot be representative samples as most organs are not uniform throughout in structure or function.—M. B.

KONWALER, B. E. [St. Mary's Hosp., Pueblo, Colo.] METASTATIC MELANOMA WITH LYMPHATIC LEUKEMIA BLOOD PICTURE. Am. J. Clin. Path., 11:761-765. 1941.

Presentation of a case, which may be one of association of 2 diseases rather than a leukemoid reaction to extensive metastasis of the melanoma.—H. G. W.

LUCIA, S. P. [Univ. of California Med. Sch., San Francisco, Calif.] LEUKEMIA: EVALUATION OF THE THERAPY. California & West. Med., 55:119-123. 1941.

A general review is given of the indications and effectiveness of chemical agents and radiation on leukemia.—M. J. E.

POPP, W. C., and C. H. WATKINS. [Mayo Clinic, Rochester, Minn.] ROENTGEN THERAPY FOR CHRONIC MACROLYMPHOCYTIC AND MESOLYMPHOCYTIC LYMPHATIC LEUKEMIA. Radiology, 37:160-162. 1941.

In reviewing the blood picture of 23 patients with chronic lymphatic leukemia who exhibited great sensitivity to roentgen therapy the authors found that the large majority of the lymphocytes were of macrolympho-

cytic or mesolymphocytic type in contrast with the usual preponderance of microlymphocytes and mesolymphocytes. These large cells did not show the nuclear immaturity which would classify them with the lymphoblasts of acute leukemia. The term "chronic macrolymphocytic and mesolymphocytic lymphatic leukemia" is proposed to describe what the authors consider to be a phase rather than a new type of leukemia.

Roentgen therapy in these cases is often followed by a radical fall in the leukocyte count and a rise in the blood urea. Doses of radiation should not exceed 100 to 125 r per field. Initial treatment of the kidney regions favors elimination in cases of renal involvement. Frequent determination of blood urea is considered of equal importance with leukocyte counts in controlling therapy.—C. E. D.

VIETA, J. O., and L. F. CRAVER. [New York, N. Y.] INTRATHORACIC MANIFESTATIONS OF LYMPHOMATOID DISEASES. Radiology, 37: 138-158. 1941.

The authors call attention to the frequency of intrathoracic involvement in Hodgkin's disease, lymphosarcoma, chronic leukemia, and mycosis fungoides. The literature is reviewed and tabulated. 1191 cases, with 160 autopsies from the Memorial Hospital are presented. Seventeen roentgenograms and 8 tables illustrate the appearance and incidence of thoracic involvement. Invasion of mediastinal and peribronchial lymph nodes is the commonest lesion in the chest but the pleura and parenchyma of the lung are frequently involved. Cavitation of the lung and bronchoesophageal fistula in Hodgkin's disease are described. Carcinoma of the gastrointestinal tract in association with lymphomatoid disease was present in 4 of 160 autopsies. Among the autopsied cases, intrathoracic involvement was found as follows:

Hodgkin's disease 88%, lymphosarcoma 73%, lymphatic leukemia 84%, myelogenous leukemia 48%. Roentgenographic study of 794 cases failed to reveal such a high incidence. The authors conclude that intrathoracic involvement is more common than is generally recognized and often cannot be diagnosed during life. More frequent roentgenograms, would, however, permit earlier detection and treatment of most lesions.—C. E.D.

Adrenal

CRAIG, L. G. [Huntington Memorial Hosp., Pasadena, Calif.] PRIAPISM FROM HYPERNEPHROMA METASTASES IN THE CAVERNOUS BODIES. California & West. Med., 55:135-139. 1941.

Progressively increasing painful enlargement of the penis in a man of 72 years developed 10 days after extirpation of a kidney involved by hypernephroma. He survived 2 months and a necropsy disclosed complete invasion by metastatic tumor of the corpora cavernosa associated with widespread deposits in the body.—M. J. E.

Pancreas

BERK, J. E. [Univ. of Pennsylvania Graduate Hosp., Philadelphia, Pa.] DIAGNOSIS OF CARCINOMA OF THE PANCREAS. Arch. Int. Med., 68: 525-539. 1941.

A discussion of the diagnostic features, with particular emphasis on the common error of the belief that a painless jaundice is characteristic of pancreatic carcinoma. Pain was found to occur earlier and more frequently than jaundice and was the outstanding symptom. Painless jaundice occurred relatively infrequently. Derangements in carbohydrate metabolism, abnormalities in roentgenograms, and elevated concentration of pancreatic enzymes in the blood are findings of invaluable aid in diagnosis of carcinoma of the pancreas.—H. G. W.

BRUNSCHWIG, A. [Univ. of Chicago, Chicago, Ill.] LARGE ISLET-CELL TUMOR OF THE PANCREAS. Surgery, 9:554-560. 1941.

This is the largest islet-tumor yet reported. It involved the tail and body, measured 15 x 13 x 10 cm. and weighed 673 gm. Only the head and 2 cm. of the neck of the pancreas remained after surgery. A slight diabetes resulted, but the patient was well 11 months after operation. The benign or malignant nature of the tumor could not be established. The nature of the operation is shown in diagram.—A. M.

FRIEDENWALD, J., and T. H. MORRISON. [Baltimore, Md.] PRIMARY CARCINOMA OF THE HEAD OF THE PANCREAS. Rev. Gastroenterol., 8:400-407. 1941.

Clinical study of 40 cases.—H. G. W.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27151. New England J. Med., 224:659-660. 1941.

A case report of a 29-year-old woman who was subject to attacks of loss of consciousness for 3 years. She has been well since resection of her pancreas for an adenoma of islet cells.—A. M.

WINTERS, W. L., P. GOTTARDO, and R. W. McNEALY. [Cook County Hosp., Chicago, Ill.] SEVERE HYPOGLYCEMIA DUE TO ISLET ADENOMA OF THE PANCREAS WITH SURGICAL CURE. West. J. Surg., 49:488-492. 1941.

Excision of a tumor in the tail of the pancreas effected an immediate cure.—M. J. E.

Pituitary

STARR, P., and L. DAVIS. [Northwestern Univ. Med. Sch., Chicago, Ill.] ENDOCRINE STUDIES OF PATIENTS AFTER SUBTOTAL HYPOPHYSECTOMY. Ann. Surg., 113:778-790. 1941.

Endocrine studies were performed on 25 patients operated upon for pituitary tumors. In 18 of this group signs of hypopituitarism were present before and after operation. The other 7 cases had evidences of hyperpituitarism. Seven illustrative case records are given. The main features of all the cases are given in 2 tables.—A. M.

Thymus

GILLESPIE, B. [Evanston Hosp., Evanston, Ill.] THYMOMA IN MYASTHENIA GRAVIS. Arch. Path., 32:659-663. 1941.

To the 58 cases of thymus gland tumor reported in myasthenia gravis is added another. In those cases of myasthenia gravis in which necropsy has been performed a benign tumor, predominantly epithelial, has been found in 50%. The significance of these epithelial tumors in the etiology of myasthenia gravis is discussed.—H. G. W.

HELLWIG, C. A. [St. Francis Hosp., Wichita, Kans.] MALIGNANT THYMOMA. Am. J. Clin. Path., 11:730-732. 1941.

Brief analysis of 8 cases, including 2 cases of Hodgkin's disease.—H. G. W.

Miscellaneous

HANCOCK, J. D. [Univ. of Louisville Sch. of Med., Louisville, Ky.] MESENTERIC TUMORS. South. Surgeon, 10:569-574. 1941.

Two cases are recorded. Reoperation on the first patient about 2 years after successful marsupialization of a cyst attached to the transverse colon disclosed a second mass in this region. When extirpated, it proved to be a cystic adenocarcinoma whose origin could not be ascertained. It did not recur. A lymphangioma situated in the mesentery of the jejunum in the second patient was removed without difficulty. Two photographs are reproduced—M. J. E.

HARVEY, W. F., E. K. DAWSON, and J. R. M. INNES. [Research Lab. of the Roy. Coll. of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh, and South-East Scotland, and the Inst. of Animal Path., Univ. of Cambridge, Cambridge.] DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY. Oliver and Boyd, Edinburgh. vii, 124. 1940.

This monograph is based on a series of papers which originally appeared in the *Edinburgh Medical Journal*. Its nine chapters, constructed on a uniform scheme of (1) definition, (2) description, (3) discussion, and (4) conclusion, deal in turn with seminoma, granulosa-cell tumor of the ovary, "mixed tumours" of salivary glands, melanoma, "lymphoepithelioma", giant-cell tumor of bone, endothelioma, lymphosarcoma, and meningioma.

While some of the opinions expressed are unorthodox (particularly in the section on giant-cell tumors in bone), the work is of great interest both from the systematic and practical aspect. Its value is further enhanced by an excellent series of photomicrographs, by the clinical and histological notes which accompany them, and by a useful bibliography of directional literature.—A. H.

Cancer Control and Public Health

GODFREY, E. S., Jr. [New York State Dept. of Health, Albany, N. Y.] NEWS RELEASE FROM DIVISION OF CANCER CONTROL, NOV. 10, 1941.

Evidence pointing to a beginning decline in cancer mortality among females in upstate New York appears from a preliminary analysis of mortality records for the period 1929 to 1940. The analysis is based on comparison of age-standardized recorded mortality rates, utilizing the 5% sample estimated population of the 1940 census. The observed decline is not great—approximately 3% when the mean annual mortality for 1929-31 is compared with that for 1938-40. However, it represents a reversal in trend and is in contrast with an increase of about 12% in the age-standardized mortality rate among males between the same periods. The population data are available only in broad age groups but on this basis the analysis indicates the observed decline to be most marked in females under 65 years of age.

Of interest in this connection is Gover's exhaustive study (Gover, M. Cancer Mortality in the United States. I. Trend of Recorded Cancer Mortality in the Death Registration States of 1900 from 1900 to 1935. Pub. Health Bull. No. 248, Washington, 1939.) of the trend of cancer mortality in the death registration states of 1900, to which group upstate New York contributes a little less than one-fifth the population. For the decade 1920-1930 her study showed a decline in cancer mortality among females age 35-44 years, but an increase in all other age-groups and in total (age-standardized) cancer mortality for each sex.

The significance of the apparent decline in female cancer mortality in upstate New York is, of course, doubtful and it remains to be seen whether it represents the beginning of a continued downward trend or only a temporary remission in the hitherto upward course of cancer mortality in both sexes.

NINETEENTH JOINT MEETING OF THE ASSOCIATION OF CONNECTICUT TUMOR CLINICS. Connecticut M.J., 5: 744-754. 1941.

Papers on carcinoma of the uterus, of the bowel, and of the breast are presented, followed by case reports of liposarcoma, malignant synovioma, lipoma, and malignant neurofibroma.—G. De B.

ROBNETT, D. A. [Columbia, Mo.] THE CANCER PROBLEM IN MISSOURI. J. Missouri M.A., 38:286-287. 1941.

Administrative details and educational activities are discussed.—M. J. E.

THOMPSON, F. G., and G. T. BLOOMER. [St. Joseph, Mo.] MISSOURI STATE CANCER HOSPITAL NO. 2: A GROUP CLINIC. J. Missouri, M.A., 38:284-286. 1941.

An account is given of the activities in 1938-39 of a cancer hospital into which 234 patients were admitted for routine diagnosis and treatment.—M. J. E.